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THE TRANSMISSION OF TRAINS OF IMPULSES THROUGH A SYMPATHETIC GANGLION AND IN ITS POSTGANGLIONIC NERVES

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The experiments reported in this paper were undertaken in order to determine the rôle of ganglionic synapses in transmitting those trains of impulses which constitute the normal activity of the sympathetic nervous system. We have also investigated certain persistent modifications in the synaptic mechanism which are induced by a series of impulses and by alterations in blood flow.

METHODS. In the course of previous work (Bronk, Ferguson, Margaria and Solandt, 1936) it had become apparent that the stellate ganglion of the cat would be an admirable preparation with which to investigate ganglionic transmission because the considerable length of postganglionic nerve makes possible an accurate determination of the form of the action potential which we have used as a sign of the discharge from the ganglion. In this respect it is more satisfactory than the commonly employed superior cervical ganglion which has a much shorter postganglionic nerve.

Most of the experiments were performed under light nembutal anesthesia; a few on decerebrate preparations. In agreement with the findings of Eccles (1935a) there were no observable differences between these two series to indicate the introduction of abnormality by the nembutal. Throughout an experiment the animal was in a moist operating chamber kept at a temperature of 34 to 36°C.

The chest wall was removed to the sixth rib and the animal placed on artificial respiration. Carefully avoiding any injury to the ganglion or its blood supply, the inferior cardiac nerve which arises from the stellate ganglion was freed from the surrounding tissues and sectioned close to the

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heart. Thus there was made available 4 to 6 cm. of postganglionic nerve in which the action potentials were recorded through silver, silver-chloride brush electrodes leading to a direct-coupled amplifier and a Matthews, General Electric or cathode-ray oscillograph.

The preganglionic nerve trunk was cut below the fourth thoracic ramus, and the first to the fourth rami were transected. The trunk was then stimulated between the second and third or between the third and fourth roots. This was done by means of silver wire electrodes connected to the secondary of a transformer the primary of which was energized by the discharge of a condenser controlled by a thyratron. A preganglionic volley was thus initiated, and this in turn produced a post-synaptic response which was recorded from the postganglionic nerve.

RESULTS. *The form of the action potential in postganglionic nerve.* It soon became apparent that, if this discharge of impulses were to be used successfully as an index to ganglionic activity, it would be necessary to know the form of the postganglionic action potential uninfluenced by any modifications which might be imposed on it by transmission through the ganglion. We have therefore stimulated the postganglionic nerve directly and recorded the response thus initiated.

Such potentials had similar form and character regardless of whether or not the nerve was in connection with the ganglion. There was accordingly no evidence either of backfiring from the ganglion or of reflex activity in the ganglion resulting from stimulation of afferent fibers. The latter possibility was further tested by splitting the postganglionic nerve into two parts, each of which remained in connection with the ganglion. Stimulating one and recording from the other gave no evidence of reflex activity, either axonal or synaptic, within the ganglion.

Following the stimulus artifact there are two spike potentials, one of which is due to the activity of many slowly conducting fibers and the other to relatively few fibers with higher conduction velocities. The latter are presumably afferent because their spike was not seen in four preparations which were deafferented by previous removal of the dorsal root ganglia T_1 to T_5, with two weeks allowed for degeneration. These impulses are therefore of no direct concern to us in the present investigation and do not appear in records obtained with the amplification generally employed.

The intervals between the stimulus artifact and the beginning and end of the spike potential under investigation correspond to conduction velocities at 36.0°C. which range from 1.4 m.p.s. for the fastest fibers down to a lower limit of about 0.6 m.p.s. These fibers must therefore belong to the C classification.

A comparison of the spike potential at various distances from the cathode indicates a considerable degree of temporal dispersion even with

relatively short conduction distances. This is characteristic of fibers with low conduction velocities (cf. Gasser, 1936) and is obviously due to the fact that a given percentile difference in velocities among such fibers gives a much greater temporal dispersion than among rapidly conducting fibers. For instance impulses travelling 10 cm. over fibers whose conduction velocities range from 100 m.p.s. to 50 m.p.s. would undergo a dispersion of 1 msec. while in fibers with conduction velocities ranging from 1 m.p.s. to 0.5 m.p.s. the dispersion would be 100 msec. During rapidly repeated activity the conduction velocity is slowed considerably, and the degree of temporal dispersion is accordingly still further increased.

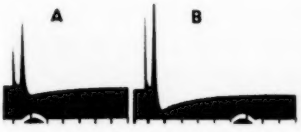

Fig. 1

Fig. 2

Fig. 1. *A*. Action potential in postganglionic nerve (inferior cardiac) from stellate ganglion elicited by a single shock to nerve (indicated by initial vertical line). Conduction distance 8 mm. Response at second electrode suppressed by cocaine. *B*. Same as *A* but with twice the amplification to show more clearly the positive after-potential. Time: 0.02 sec.

Fig. 2. Development of negative after-potential during repetitive stimulation. *A*, at the beginning of the train. *B*, after 5 sec. stimulation at 6 per sec. Time: 0.05 sec.

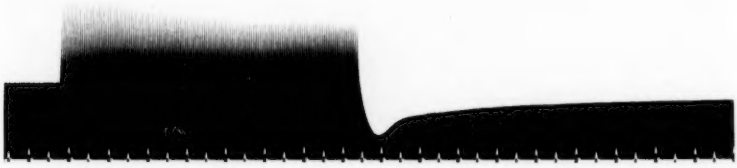

Fig. 3. Positive after-potential following 3 sec. tetanus at 46 per sec., 2 cm. conduction distance. Time: $\frac{1}{5}$ sec.

In freshly excised nerve which has not been stimulated for some minutes the typical response at a single lead consists of a positive after-potential following immediately after the spike. There is little or no after-negativity. See figure 1. This is in agreement with the observations on C fibers in other nerves by Bishop (1934), by Richards and Gasser (1935), and by Gasser (1936), as is also the observation that the after-potential in these fibers is larger relative to the spike than in the A fibers studied by Gasser (1935) and by Gasser and Grundfest (1936). Even this potential is small, however, compared with the after-positivity revealed at the end of a train of impulses as in figure 3. The magnitude of this positive

after-potential is greater the longer the duration and the higher the frequency of the previous stimulation, within certain limits.

In our earlier experiments we frequently observed a large negative after-potential continuing out of the spike in a single nerve action which followed a period of rest, although seldom in freshly excised nerve. Latterly, however, we have employed more care in keeping the nerve in good condition, and in these experiments we have found marked after-negativity much less frequently in the rested nerve. We are not able to define all of the conditions responsible for a large negative after-potential but deterioration of the nerve certainly favors its production (see also Gasser, 1935). Of more significance to the present investigation is the fact that it invariably develops during the course of a train of rapidly repeated impulses (cf. fig. 2), in some cases attaining a magnitude nearly as great as that of the spike potential. Under these conditions the negative after-potential may obscure the development of the positive after-potential during the course of the stimulation, and finally it intervenes between the last spike and the after-positivity at the end of the tetanus.

The conditions or processes in a nerve which cause a large after-negativity to appear in the action potential persist for a considerable time following a period of repeated stimulation. If, for instance, a considerable negative after-potential has been developed by a series of stimuli repeated at the rate of 5 or more per second, a single response after a rest interval of some seconds has a negative after-potential much larger than that observed at the beginning of the initial period of stimulation. Only gradually and over a period of many seconds does the condition of the nerve which is responsible for the development of the large after-negativity disappear. This is illustrated in figure 4. *A* is the response to a single shock. *B* is the last action-potential of a 45 sec. tetanus at the rate of 12 a sec. and shows a large negative after-potential. *C*, *D*, *E* and *F* are the responses to single stimuli at 5, 10, 20 and 40 sec. after the end of the tetanus.

There is likewise a persistence of that condition which causes the production of a large positive after-potential. For many seconds following the end of a prolonged period of stimulation a short series of stimuli will evoke a much larger after-positivity than would follow such a series in nerve which had not been previously stimulated. In figure 5 for instance *A* represents the end of a 1 sec. tetanus and shows only a relatively small positive after-potential. In *B* the nerve had been stimulated for 10 sec., and there is a large after-positivity. *C*, *D* and *E* are as *A* at the end of short 1 sec. tetani 2, 15 and 60 sec. after the end of the prolonged period of stimulation. Throughout this period there is a larger positive after-potential than in the control.

A knowledge of such cycles of potential change in the postganglionic

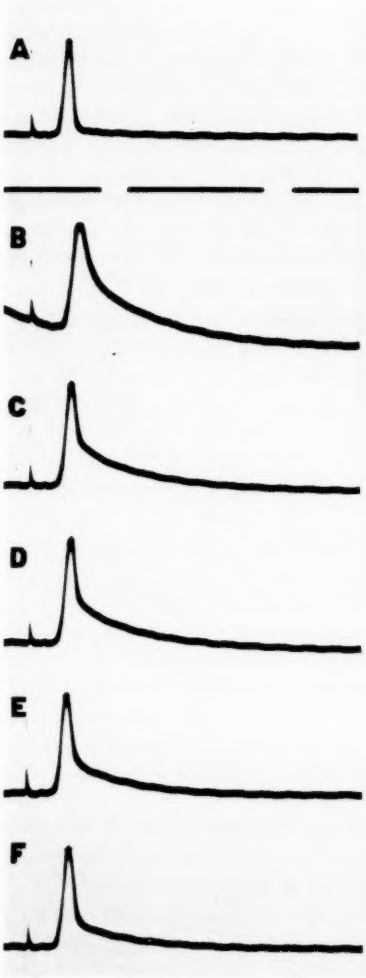

Fig. 4

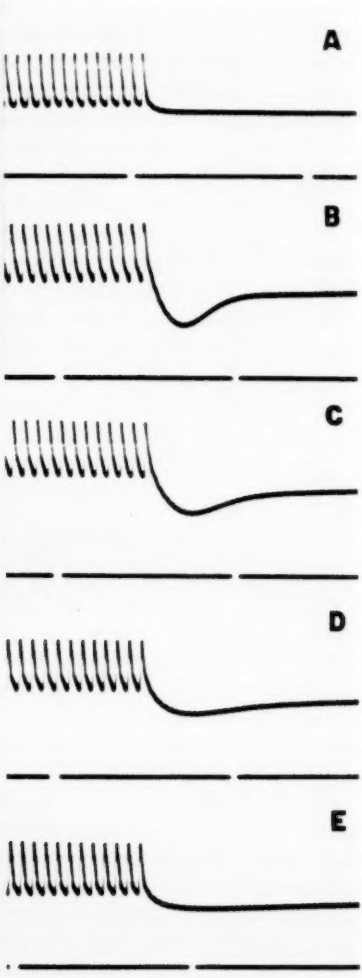

Fig. 5

Fig. 4. Negative after-potentials before and after tetanization. *A*, response in rested nerve. *B*, last of a train of impulses at 12 per sec. for 45 sec. The nerve is then rested with single test stimuli at *C* 5 sec., *D* 10 sec., *E* 20 sec. and *F* 40 sec. after the end of the tetanus. Showing persistence of capacity for developing negative after-potential. Time in *A* and all others, 0.1 sec.

Fig. 5. Positive after-potential following short tetani before and after a period of prolonged stimulation. *A*, the end of 1 sec. tetanus at 15 per sec. in rested nerve. *B*, the end of 10 sec. tetanus. *C*, *D* and *E* are at the end of 1 sec. tetani 2, 15 and 60 sec. after the 10 sec. tetanus. Showing gradual decrease in capacity for developing positive after-potential. Time: 0.5 sec.

nerves is important in a consideration of ganglionic activity for several reasons. We have suggested that such knowledge is essential if the postganglionic potentials are used in analyzing the discharge of the ganglion cells. For instance, an action potential showing prolonged negativity might be interpreted as evidence of after-discharge from the ganglion, if the protracted negativity were not recognized as an after-potential. Second. Because of the important correlation between the potential cycle and the irritability of the nerve (cf. Gasser and Grundfest, 1936) it is highly probable that a determination of the potential changes in the intraganglionic post-synaptic fibers and in the ganglion cells may give valuable clues as to the cycles and mechanisms of ganglionic irritability and excitation. Third. We will subsequently describe long lasting changes in the response of ganglion cells which are the result of trains of preganglionic impulses. It is not unlikely that such changes are related to the persistent modifications in the property of an axon which cause the development of a larger after-potential many seconds after the end of a tetanus, as described above. And lastly, the nature of postganglionic fiber potentials must be seriously considered in any interpretation of ganglionic potentials.

In a preliminary note (Bronk, Tower and Solandt, 1935) we have raised the question as to whether the slow potentials recorded by Eccles (1934) from the superior cervical ganglion can be attributed to the activity of the intraganglionic nerve fibers and not primarily to the ganglion cells. By delaying this publication, however, we have had an opportunity to see the records on which Eccles' preliminary reports were based. Those records (Eccles, 1935c) and our own observations of ganglion potentials show in the case of a single and unrepeated volley a slow positive wave with an amplitude which is larger relative to the spike potential than any we have seen in a rested postganglionic nerve from the stellate ganglion. We have therefore at present no evidence against Eccles' view that the slow potential waves in the ganglion are cell body potentials. On the other hand we feel that conclusive support for that hypothesis is not now available. For instance certain conditions such as temporal dispersion, a diffuse lead through inactive tissue, and the chemical environment of the fibers (Lehman, 1937) accentuate the size of the after-potential in nerve fibers relative to the spike potential. Some of these factors may, in the ganglion, differ from post-ganglionic nerve and thus give rise to relatively larger after-potentials (cf. Bishop, 1936). Mere size of after-potential therefore would not be an adequate criterion for identifying a cell potential.

Postganglionic response to a preganglionic volley. Each volley of preganglionic impulses entering the ganglion causes the discharge of a single group of postganglionic impulses, and there is accordingly no evidence of an after-discharge from the ganglion in the form of repetitive, syn-

chronized bursts of impulses. This is in agreement with the findings of Bishop and Heinbecker (1932). For reasons which will presently appear, however, such evidence does not settle the question as to whether there is a repetitive discharge from the individual ganglion cells.

The spike potential of a postganglionic volley initiated by a preganglionic stimulus (cf. fig. 7*B*) may have a duration of 20 msecs. even though the lead is just distal to the ganglion and the preganglionic nerve is stimulated immediately proximal thereto. Three possible factors may contribute to this temporal dispersion introduced by the ganglion. A. Because of the scattered positions of the cells in the ganglion some impulses may traverse the 5 or 10 mm. length of the ganglion over slowly conducting postsynaptic fibers, others over rapidly conducting presynaptic fibers (cf. Bishop, 1936). B. Synaptic latencies for the several units may differ. C. The individual ganglion cells may discharge repetitively in response to a single preganglionic volley.

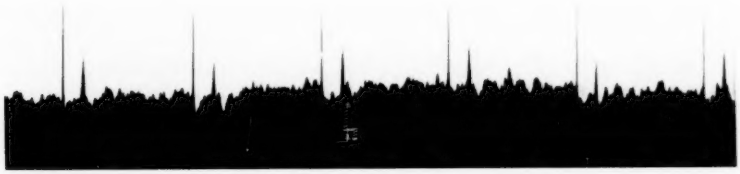

Fig. 6. Discharge in a single or in a few fibers of postganglionic nerve responding to preganglionic stimulation. Shows no repetitive discharge. Marker represents stimuli at 0.2 sec. intervals.

This last question of whether a ganglion cell responds more than once to an incoming volley has been investigated with indirect methods by various workers. For instance, by comparing the contractions of the nictitating membrane initiated by stimulation of the pre- and of the post-ganglionic fibers of the superior cervical ganglion Knoeffel and Davis (1933) and Brown (1934) have obtained strong presumptive evidence that a single preganglionic stimulus gives rise to a single volley of impulses in the postganglionic fibers. More direct and conclusive evidence should be obtainable by the methods employed in the present investigation.

This has been done by isolating and recording from one or a very few postganglionic fibers. To do so is difficult because of the small size of the fibers and the way in which they are bound together in the nerve trunk, but in four experiments we have succeeded in reducing the number of fibers to such an extent that the response consisted of a single, sharp spike for each preganglionic stimulus, as in figure 6. There is no indication of after-discharge. On the basis of such evidence we conclude that a ganglion cell does not normally respond repetitively to a single preganglionic volley.

Quite different results are obtained in the case of invertebrate ganglia for there a single volley going into the ganglion may set up a repetitive discharge in a postganglionic fiber (Bronk and Pumphrey). Similarly a single afferent volley may initiate a repetitive discharge from an anterior horn cell of a vertebrate (Adrian and Bronk, 1929). But these differences in the character of the response, compared with that of a sympathetic ganglion, do not necessarily indicate a difference in synaptic mechanisms. For, although it has been suggested by some that the excitatory state

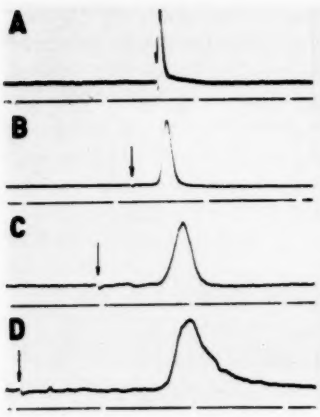

Fig. 7

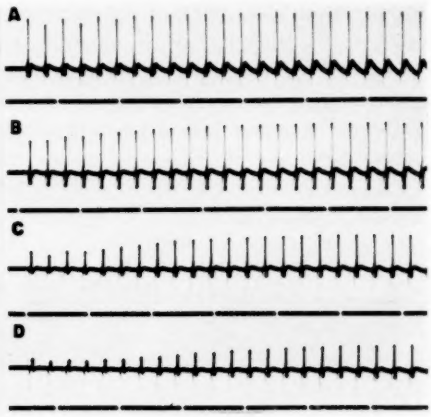

Fig. 8

Fig. 7. Temporal dispersion along sympathetic pathways. Preganglionic nerve stimulated 13 mm. from ganglion. *A*. Preganglionic response, 13 mm. conduction distance. *B*. Postganglionic response 6 mm. beyond ganglion. *C*. Same at 30 mm. beyond ganglion and *D*, 60 mm. from ganglion. Amplification adjusted to give constant spike heights. Time: 0.05 sec.

Fig. 8. Build-up of postganglionic response in non-circulated ganglion due to recruitment of ganglion cells during repetitive stimulation of preganglionic nerve (maximal stimuli to one root). *A*. Control with circulated ganglion. Circulation then cut off (ganglion excised). Subsequent records *B*, 7 min., *C*, 16 min. and *D*, 25 min. thereafter. Time $\frac{1}{2}$ sec.

resulting from a single afferent volley may persist long enough to cause repetitive firing from the motor nerve cell, the existence of internuncial neurones in the central nervous system of vertebrates and in the ganglionated cord of invertebrates makes possible other explanations of the after-discharge on the basis of delayed paths (Forbes, 1922) or chains of neurones (Lorente de Nó, 1933). At the present time there is to our knowledge no evidence that a synchronized volley of impulses arriving at a motoneurone initiates the discharge of more than a single postsynaptic impulse—at least with frequencies of excitation and conditions of circulation normally

existing in the organism. Our findings concerning synaptic mechanisms in sympathetic ganglia are in accord with that general statement.

How much of the temporal dispersion introduced by the ganglion is due to different synaptic latencies and how much to differences in the character of intraganglionic fiber paths traversed by the several impulses (see A and B above) can only be estimated. Assuming that some impulses traverse the whole length of the ganglion (say 1 cm.) over postsynaptic fibers having a conduction velocity of 0.5 m.p.s. and others the whole length over presynaptic fibers with a conduction velocity of 10 m.p.s. we obtain a maximum figure for temporal dispersion of about 20 msecs. This is adequate to account for all of the dispersion which appears on conduction through the ganglion, and it is therefore not necessary to assume that differences in synaptic latency are responsible in any appreciable degree.

The temporal dispersion of impulses which occurs in a ganglion continues to develop to a marked degree as the impulses travel along their postganglionic pathways. As pointed out in the previous section this is characteristic of groups of slowly conducting fibers. It is well illustrated in figure 7. The uppermost record is of the preganglionic action potential with a conduction distance of 13 mm. In the subsequent records there is the same preganglionic conduction distance as for the first, but the leads are from the postganglionic nerve 6 mm., 30 mm. and 60 mm. distal to the ganglion. Because of the inertia of the effector systems supplied by the sympathetic fibers, it is doubtful whether there is any functional significance associated with the considerable spread in times of arrival of impulses which started synchronously in a grouped discharge. In the somatic system, on the other hand, such a temporal dispersion of the impulses arriving at a rapidly acting skeletal muscle would interfere with the execution of short, quick movements, and a large temporal dispersion in sensory nerves would reduce the capacity of the organism for perceiving the rapid flicker of lights or the movement of vibrating bodies.

On the basis of such evidence as is presented in this section we may conclude that the function of a sympathetic ganglion is to transmit impulses from the preganglionic to the postganglionic fibers without altering their frequency or the characteristic grouping which has been observed by Adrian, Bronk and Phillips (1932), Bronk, Ferguson, Margaria and Solandt (1936), and Govaerts (1936). Nor is the temporal dispersion developed in the ganglion large compared with that due to conduction along the postganglionic fibers. The most significant influence of the ganglion still appears to be the introduction of a large amount of spatial disperson by delivering a single preganglionic impulse to many postganglionic fibers and thus to a wider motor area (cf. Billingsley and Ranson, 1918).

Ganglionic response to repetitive stimulation and effects of altered circulation. In the characteristic response of a ganglion to a low-frequency train of preganglionic volleys, either maximal or submaximal, the successive postganglionic spike potentials are of fairly uniform magnitude. This indicates that a constant number of ganglion cells is responding. Under certain conditions, however, we have observed a progressive increase in the amplitude of the spike potential during the course of repetitive stimulation. In such experiments submaximal stimulation of the preganglionic trunk, or maximal stimulation of one of the roots, at a rate of from 3 to 15 per sec. gave a postganglionic response which became progressively larger until, after 40 or 50 stimuli, the spike potential had increased 3 to 6 fold. The increased potential is due to a recruitment of additional ganglion cells because we do not find such an increase in the response of either the preganglionic or postganglionic nerves when they are stimulated directly at these frequencies.

While searching for the conditions which cause such a progressive "build-up" of the postganglionic response we found that it was almost always present, and to a large degree, when the ganglion was excised. This suggested that the recruitment of cells is in some way due to inadequate circulation, and many experiments have supported that conclusion. The increased response has been observed infrequently when the ganglion had not been disturbed, but there remains the possibility that in those cases the circulation was defective due to injury or some other cause.

A typical experiment which illustrates the effect of altered circulation is shown in figure 8. The preganglionic nerve to a normal ganglion was stimulated at the rate of 7 per sec., and in each of many such tests the size of the last postganglionic spike potential differed but little from the first. The ganglion with its pre- and postganglionic nerves was then removed from the animal and kept within a degree or two of body temperature in the warm moist-chamber. Seven minutes later the nerve to the ganglion was stimulated as before, and there was then a progressive increase in the size of the postganglionic response, the 20th being 50 per cent larger than the first. Twenty-five minutes after excision of the ganglion the effect was even more marked with a 3 fold increase in the amplitude of the spike potential during the course of the stimulation. Thus the capacity for recruitment increases progressively for 20 minutes or more after excision, as is further illustrated by figure 9 (A). Recruitment is therefore dependent on changes developing gradually in the absence of circulation. During the same period of time the magnitude of response elicited by a single preganglionic volley of constant intensity becomes progressively smaller (fig. 9B). The capacity for recruitment thus increases as the ganglion cells lose their ability to respond to a single, unrepeated stimulus.

An understanding of the factors responsible for this phenomenon and that described in the succeeding section will undoubtedly add much to

our knowledge of synaptic mechanisms. At the present time however we are unable to do more than suggest possible mechanisms in terms of current concepts. We may assume that in the absence of circulation some seconds are required for the disappearance of the excitatory state developed within the ganglion by each preganglionic volley, and that there is accordingly a summation of the effects produced by the successive volleys in a train. Thus the level of the excitatory state is progressively raised, and cells are brought into action which do not discharge impulses in response to a single incoming volley. The usual absence of such recruit-

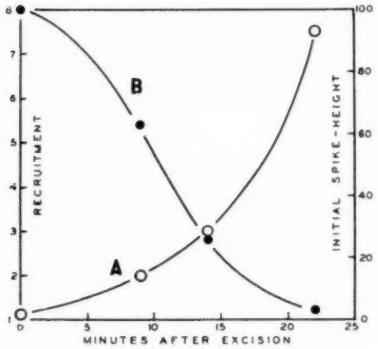

Fig. 9

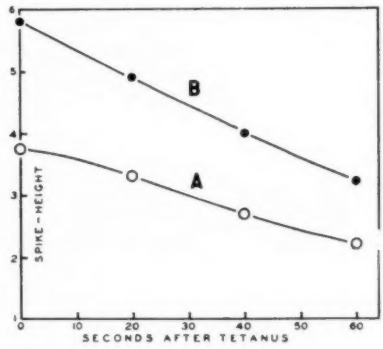

Fig. 10

Fig. 9. *A*. Recruitment during trains of impulses 9, 14, and 22 minutes after excising the ganglion. Degree of recruitment represented as ratio of the amplitude of last postganglionic spike in each train to the first. One preganglionic ramus stimulated maximally for 5 sec. at 6 per sec. *B*. Progressive failure of ganglion to transmit single volleys following excision. Ordinates give height of initial spike in each of the trains described in the *A*.

Fig. 10. Increased response of non-circulated ganglion to preganglionic volleys following a period of activity. *A*, 17 min. and *B*, 37 min. after excision. In each case preganglionic nerve was first stimulated for 10 sec. at 7 per sec. Test shocks were then applied 20, 40, and 60 sec. after end of tetanus. Ordinates give spike heights as multiples of the height of initial spike in the tetanus.

ment in a normally circulated ganglion, on the other hand, may be due to a rapid decline of the excitatory state, or to the production by even a single volley of an excitatory state which is supraliminal for most of the postganglionic cells innervated by those preganglionic fibers which are stimulated.

The factors which underlie these phenomena may be related to those responsible for the facilitation of the second of two preganglionic volleys described by Eccles (1935b) but the persistence of the excitatory state with which we are dealing is very much longer than any he has reported.

Long persistence of excitatory state in ganglia. Even the duration of the excitatory state indicated in the previous paragraph is small compared with that revealed by the following typical experiment. The preganglionic nerve to an excised or non-circulated ganglion was stimulated at the rate of 7 per sec. for 10 sec. with the usual "build-up" (about 6 fold) of the postganglionic response due to the recruitment of ganglion cells. When 20, 40 and 60 sec. later the preganglionic nerve was again stimulated with single shocks of the same strength the postganglionic response was still 5, 4 and 3.2 times greater than the first response of the conditioning series (fig. 10B). During the course of repeated activity a condition develops in the ganglion as a result of which more ganglion cells respond to a given preganglionic volley. Only gradually does this condition disappear. Indeed we have frequently observed such an augmented response 90 sec. after the end of the previous activity. At the present time we are unable to do more than describe the phenomenon and say that the magnitude of the effect is greater the longer the period of stimulation.

The effects of acetylcholine on ganglionic discharge. In an effort to relate the above observations to the neuro-humoral theory of synaptic transmission we have made many experiments concerning the effects of acetylcholine on the activity of ganglion cells. Although this work is still in a preliminary stage certain observations are sufficiently definitive to report.

In order to control the action of the drug the stellate ganglion was perfused, and this we have done in the following manner. The axillary artery was cannulated so that the perfusion fluid, which was Locke's, flowed back into the subclavian artery. The thyrocervical axis, and the mammary and vertebral arteries were ligated at their points of departure from the subclavian, and the subclavian was clamped just caudal to the vertebral artery. The perfusion fluid then reached the ganglion through a fine branch of the costocervical axis although some fluid was lost to other parts supplied by this vessel.

When about 1 cc. of acetylcholine made up to a concentration of 1:10,000 in Ringer's fluid was injected into the cannula through which the perfusion fluid was flowing there was a random discharge of impulses from the ganglion cells, as shown in figure 11A. This is in agreement with the observations of Feldberg and Gaddum (1934) and Feldberg and Vartiainen (1934) that acetylcholine perfused through the superior cervical ganglion causes contraction of the nictitating membrane. In occasional experiments (fig. 11B) there was in the discharge a fairly regular series of spikes representing the rhythmic discharge from a single ganglion cell or from a group of cells firing synchronously.

If, during submaximal, low frequency stimulation of the preganglionic nerve, acetylcholine is injected into the perfusion fluid as described in the previous paragraph, the size of the postganglionic volleys is largely in-

creased. Such a potentiating effect of the acetylcholine is well illustrated in figure 12. We would suggest that it may be due to a lowering of the threshold of the ganglion cells by the acetylcholine so that a given number of preganglionic impulses which were previously unable to activate certain cells now excite them to discharge. Or, if synaptic transmission is normally accomplished by the liberation of acetylcholine from the preganglionic terminations, it may be argued that the acetylcholine in the perfusion fluid

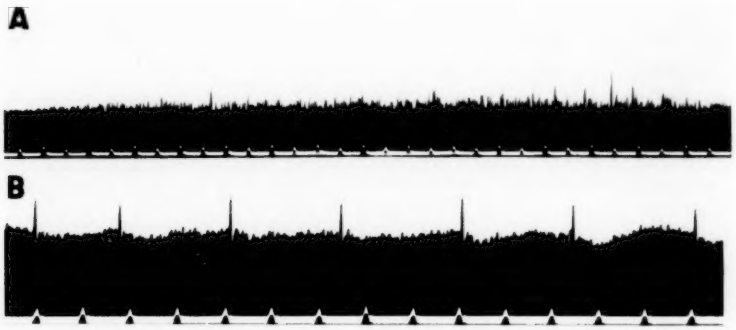

Fig. 11. Discharge from ganglion perfused with acetylcholine bromide. *A*. Typical random discharge. *B*. Rhythmic discharge from one or a few ganglion cells. Time: $\frac{1}{5}$ sec.

Fig. 12. Increased discharge from ganglion in response to sub-maximal preganglionic volleys during perfusion of ganglion with acetylcholine. During signal 1 cc. of 1:10,000 solution injected into perfusion cannula. Last 2 records 15 and 85 sec. after injection. Time: $\frac{1}{5}$ sec.

added to that produced by the submaximal preganglionic volley raises the concentration to a level adequate to produce stimulation of cells with high thresholds. We have no evidence at hand for deciding between these alternative explanations, and it is probable that there are others equally deserving of consideration.

SUMMARY

The conduction of trains of impulses through the stellate ganglion has been studied by stimulating the preganglionic fibers and recording the action potentials in the postganglionic nerve. In order to have a basis for interpreting such evidence of ganglionic activity the form of the action

potential in response to direct stimulation of the postganglionic nerve has also been investgated.

The character of the response to postganglionic stimulation is not modified by separating the nerve from the ganglion. There is accordingly no evidence of backfiring from the ganglion or of ganglionic reflexes.

The postganglionic fibers under investigation have conduction velocities ranging from 1.4 to 0.6 m.p.s. Consequently there is a considerable temporal dispersion of the spike potential with even relatively short conduction distances.

Following the spike there is a positive after-potential which increases considerably in magnitude during the course of a tetanus.

Rested nerve in good condition does not generally show a measurable negative after-potential following a single spike. During the course of even a low-frequency tetanus however a negative after-potential invariably develops and attains a considerable size, which may at times equal that of the spike.

The conditions of the nerve which make for large positive and negative after-potentials persist for many seconds following the end of a period of repeated stimulation.

A volley of preganglionic impulses initiates a single, temporally dispersed volley of postganglionic impulses. Records from a few fibers of the nerve show that an individual nerve cell discharges a single impulse in response to each preganglionic volley.

The temporal dispersion introduced by the ganglion is primarily due to differences in conduction time for the various fiber pathways through the ganglion.

Either maximal or submaximal stimulation of the preganglionic nerve at frequencies of not over 10 or 20 per sec. sets up postganglionic discharges of constant magnitude, showing that a constant number of ganglion cells is activated.

If the circulation of a ganglion is stopped, there is a progressive decrease in the number of cells which respond to single preganglionic volleys.

In a non-circulated ganglion trains of submaximal preganglionic volleys of constant size and low frequency progressively recruit more and more ganglion cells with a consequent "build-up" of the postganglionic response. After 20 or more volleys the number of ganglion cells responding may be increased 5 to 6 fold.

After such a period of stimulation the number of ganglion cells responding to preganglionic volleys of given strength declines slowly to what it was before the tetanus. In a non-circulated ganglion there is accordingly a long persistence of the excitatory state which may last as long as 60 sec.

Perfusion of a ganglion with acetylcholine induces either a random dis-

charge of impulses—or a rhythmic discharge from single cells or closely synchronized cells.

During perfusion with acetylcholine the number of ganglion cells responding to a submaximal preganglionic volley is greatly increased.

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EFFECT OF ADMINISTRATION OF ADRENAL CORTICAL HORMONE PREPARATIONS ON FERTILITY, PREGNANCY AND LACTATION IN THE NORMAL RAT[1]

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That adrenal insufficiency causes profound disturbances in the reproductive activities of mammals and that administration of active adrenal cortical hormone preparations restores such activities is now well established. Whether a supernormal level can be evoked in animals retaining their own adrenal glands by hormone administration is less clear. Interest has centered in the claims of Corey and Britton (1931) that this treatment induced precocious sexual maturity; a result which Howard and Grollman (1934) and others, however, have been unable to confirm. The literature on this subject, together with that on the allied studies of ovarian, uterine and testicular growth and histological changes has been reviewed by Fitzhugh (1937).

The present investigation was designed to survey quantitatively the influence of large doses of the hormone given daily to sexually mature rats before mating, during pregnancy and during lactation. The rapid synthesis of organic materials occurring in gestation and lactation involves considerable quantities of water and salts, in the metabolism of which recent developments have shown the adrenal cortical hormone to play a conspicuous rôle. It was therefore deemed proper to conduct these studies under conditions in which the mineral composition of the diet was unfavorable to the organism in a direction such that excess of the hormone might be expected to exert a salutary influence. In the study on pregnancy a low-sodium-high-potassium diet was used; while in that on lactation it was sought to accentuate the adverse effect of a low sodium diet by the unusual milk demand made by enlarging the litter. Since the

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reproductive processes which could conceivably be altered by the hormone given before mating do not involve conspicuous water and salt exchanges, only a normal mineral diet was used in this part of the investigation.

GENERAL METHODS. Adult albino rats of the Slonaker-Wistar strain were used. They were housed in cages bedded with wood shavings, and provided with water and food ad libitum. The food was a powder of the following composition: corn meal (70.9 per cent), linseed oil cake (9.7 per cent), alfalfa meal (1.9 per cent), casein (9.7 per cent), biotol (4.9 per cent), bone ash (1.4 per cent) and wheat germ (1 per cent). With the addition of 0.5 per cent sodium chloride this became our "normal salt diet," of which the sodium content was 0.37 per cent and the potassium content 0.37 per cent. The original powder, our "low sodium diet," contained 0.07 per cent sodium and 0.37 per cent potassium. When to the original powder was added 0.6 per cent potassium chloride, it became our "low sodium high potassium diet," containing 0.07 per cent sodium and 0.68 per cent potassium.

Two adrenal cortical hormone preparations (hereinafter called "A.C.H.") were used: 1. Eschatin, a Swingle-Pfiffner extract prepared by Parke Davis and Company, was injected in the fertility and lactation studies. Control animals received 0.5 per cent phenol (the preservative of Eschatin) in 0.9 per cent NaCl. In the larger doses used (2 and 2.2 cc.) Eschatin frequently caused tremors and convulsions lasting several minutes. Since control injections had the same effects, they were doubtless due to the phenol preservative. 2. The Adrenal Cortex Extract prepared by the Wilson Laboratories was given in the pregnancy study. Controls received 1:100,000 merthiolate (the preservative of this extract) in 0.9 per cent NaCl. No untoward effects were observed to follow use of this preparation or its control solution.

These preparations were assayed by the rat growth method of Grollman (1936), using, however, a low salt diet. On the assumption that the quantity of a preparation yielding a mean weight increase of 1.5 grams per rat per day contained $\frac{1}{2}$ rat unit, the potencies were: Eschatin, 0.16 rat unit per cubic centimeter; Wilson's Extract, 0.40 rat units per cubic centimeter.

In computing the statistical significance of differences throughout this paper we have used Fisher's method for small numbers of cases (Fisher, 1930). "S.E." in the tables refers to the conventional standard error of the mean.

FERTILITY. Under this heading, we report experiments in which A.C.H. was administered to male and female animals before mating and its influence determined on the incidence of pregnancy and on number of embryos.

Methods. Eighty females, sexually mature but not more than two

months beyond the time of opening of the vaginal orifice, were divided into four equal groups: 1, those receiving 2 cc. Eschatin subcutaneously daily for eight days, following which they were mated with males which had received similar Eschatin treatment; 2, those receiving Eschatin mated with males which had received 2 cc. 0.5 per cent phenol solution ("control males"); 3, those receiving 2 cc. 0.5 per cent phenol solution ("control females") mated with males which had received Eschatin; and 4, control females mated with control males. At the conclusion of the injection period, small groups (two to four) of females were placed in a cage with one male for mating. Lest disability of individual males might unduly prejudice the females exposed to them the males receiving similar injections were rotated among the females to which they were assigned, being changed three times daily. The thirty-six males which were used were about two months older than the females. After a mating period of six days, long enough to permit all females with normal cycles to come into

TABLE 1

Influence of adrenal cortical hormone (Eschatin) on incidence of pregnancy and litter size

	CONTROL MALES				ESCHATIN-TREATED MALES			
	Number of females	Number pregnant	Mean litter size	S.E.	Number of females	Number pregnant	Mean litter size	S.E.
Females:								
Control	20	4	6.25	0.73	20	5	7.00	1.28
Eschatin-treated	20	6	8.33	0.37	20	10	7.50	0.54

estrus at least once, the sexes were separated. Fourteen days later, the females were killed, the abdomens opened, and the existence of pregnancy and the number and state of the embryos (if any) recorded.

Results: Incidence of pregnancy. The number of pregnancies occurring in each of the four groups is given in table 1. A.C.H. apparently increases the incidence of pregnancy as compared with that in the controls in some degree when given to one parent, and in greater degree when given to both parents. Application of the χ^2 test (Fisher, 1930) to the group in which both parents received A.C.H. as compared with the untreated controls indicated that the distribution of incidence of pregnancy observed could have occurred only 5 times in 100 had the A.C.H. been without effect.

It may be concluded that the data show a marked tendency for the treatment of sexually mature rats with A.C.H. to increase their fertility, the influence exerted on the females being more marked than that on the males. Concerning the mechanism of the action on the females, some light may be derived from the observation made in the experiments on

pregnancy (q.v.) that, in untreated animals in estrus and with post-coital vaginal plugs, pregnancy resulted in about 90 per cent of cases, as contrasted with 20 per cent in untreated animals simply placed together for six days. These facts suggest that the fundamental physiological processes involved in ovulation, fertilization and early pregnancy are already at a high level of efficiency and thus cannot well be the means of increase in fertility. The remaining processes, predominantly behavioral, such as sexual receptivity or drive, suggest themselves as more probably responsible. Studies of the estrous cycle in normal mice and rats given A.C.H. by Cleghorn (1932), by Howard and Grollman (1934) and by King (1937) failed to show any effect. Corey (1937) and Fitzhugh (1937) conclude that A.C.H. is of itself not estrogenic. However, the recent work of Dempsey, Hertz and Young (1936) on the guinea pig showing active participation by the corpus-luteum hormone in hormonal control of estrous behavior (although not supported by the observations of Ball (1936) on the rat), and the tendency of A.C.H. in normal rats to cause luteinization (literature cited by Allen and Bourne, 1936), may suggest a possible influence. Obviously, information is inadequate to permit any conclusion relative to the mechanism of the increased fertility we observed. Concerning the action on male animals, the observations of Howard (1937) and Davidson (1937) that an adrenal cortical hormone exists which can in part prevent or repair castration atrophy of the male accessory reproductive organs, and that of Ehrenstein and Britton (1937) that adrenal cortical extracts contain a sodium salt of palmitic acid which can activate testosterone, are of interest. Whether Eschatin contains these substances, and, if so, whether their administration to normal males can raise their fertilizing capacity is not known.

Litter size. Table 1 also contains the data on litter size. The hormone again appears to have exerted a favorable effect when given to the females. The difference in litter size between control and Eschatin-treated females (both served by control males) is one that would occur only 12 times in 1000 if the Eschatin treatment were without effect and is therefore quite reliable. No significant difference appears in the females served by control and Eschatin-treated males.

Whether the greater mean litter size, observed in the latter half of pregnancy, in A.C.H. treated females represents an increase in the number of ovulations, in the number of ova fertilized or implanted, or a decreased incidence of intrauterine mortality, is not known. Conceivably, if the luteinizing action of A.C.H. referred to in the previous section occurred in our animals, the resulting secretion of progesterone might have a favorable effect on survival of the embryos.

PREGNANCY. The second series of experiments sought to determine whether A.C.H. administered to pregnant females, on normal and low-

sodium-high-potassium diets, affected the duration of gestation, the number of young delivered and their weight.

Methods. Young nulliparous female rats, about one month after opening of the vaginal orifice, in estrus (as evidenced by relaxed vaginal ring and cornification) on any given evening were placed with sexually active males for three or more night hours. Those showing vaginal plugs were considered to have been inseminated, and were transferred to special cages for the duration of pregnancy. Those few (about 10 per cent) which proved not to be pregnant were discarded as soon as this fact was evident. The animals were assigned in rotation to four groups: 1, those on normal salt diet receiving 2 cc. Wilson's Adrenal Cortex Extract subcutaneously daily; 2, those on normal salt diet receiving 2 cc. merthiolate solution;

TABLE 2

Influence of adrenal cortical hormone given during pregnancy

	NORMAL SALT DIET		LOW Na-HIGH K DIET	
	Control	A.C.H.	Control	A.C.H.
Number of cases	7	6	4	5
Duration of pregnancy:				
Days	21.44	21.67	21.90	21.76
S.E.	±0.09	±0.15	±0.24	±0.20
Total litter weight:				
Grams	32.21	35.03	24.90	35.06
S.E.	±3.62	±2.25	±4.08	±2.37
Litter size:				
Number of young	7.00	7.17	5.25	7.00
S.E.	±0.91	±0.52	±1.00	±0.50
Mean weight of young:				
Grams	4.70	4.92	4.80	5.01
S.E.	±0.20	±0.24	±0.20	±0.13

3, those on low-sodium-high-potassium diet receiving 2 cc. Adrenal Cortex Extract; and 4, those on low-sodium-high-potassium diet receiving 2 cc. merthiolate. On the day before parturition was expected they were put in individual cages with wide mesh wire cloth floors through which the young fell—with the intent of preventing maternal cannibalism. The young were counted and weighed as soon as observed. Mothers' weights were determined before mating and at intervals during pregnancy. The results are presented in table 2.

Results. Duration of pregnancy. This term is used herein to mean the elapsed time between the estimated time of insemination (midtime between placing mating animals together and separating them) and the estimated time of parturition (midtime between last pre-delivery observation and

the first post-partum observation). For the average case the maximum possible error in the estimation of the duration of pregnancy is about 6 hours.

Effect of mineral content of the diet. The use of a diet low in sodium and high in potassium prolonged pregnancy from 21.44 days to 21.90 days, the difference being one that would occur 5.9 times in 100 if the diet had no effect. Since the abnormal diet resulted in a smaller total litter weight than did the normal diet, the prolongation of pregnancy duration observed may have been at least in part the result of decreased uterine distention. Orent-Keiles, Robinson and McCollum (1937) have found that sodium depletion may prolong the gestation period of the rat.

Effect of A.C.H. While on the normal salt diet the duration of pregnancy is slightly greater in the animals given A.C.H. as compared with their controls, the reverse is true on the low-sodium-high-potassium diet. In neither case is the difference statistically reliable. In the former group, the total litter weight, greater in the A.C.H. than in the control animals, should have shortened gestation, a fact which lends some slight confidence in the reality of the observed prolongation. In the latter group, the total litter-weight difference is in a direction tending to account for the observed difference in gestation time. No definite conclusion is warranted.

Total litter weight. Influence of mineral content of the diet. The low-sodium-high-potassium diet reduced the total litter weight from 32.21 grams to 24.90 grams at delivery. The difference between these means could occur 19.5 times in 100 if the diet were without effect, and is therefore not very reliable. If account, however, is taken of the fact that gestation lasted about 12 hours longer in the latter group, and a correction introduced based on a normal post-natal growth rate of 1.0 grams per young per day, which was characteristic of our colony, the estimated total litter weight for the normal diet becomes 35.41 grams, a value lending considerably greater confidence to the view that the abnormal mineral content of the second diet has depressed the growth of the embryos during gestation.

Influence of A.C.H. On the normal salt diet A.C.H. appeared to cause a slight increase in total litter weight. Correction for the slightly longer duration of pregnancy almost exactly abolishes the difference between the A.C.H. litters and their controls. However, on the low-sodium-high-potassium diet, A.C.H. produced an increase over the corresponding controls which could occur only 3 times in 100 if A.C.H. had no influence. Moreover, correction for the shorter mean duration of pregnancy in the A.C.H. litters, lowers the control value to 24.20 grams thus increasing the difference between control and treated litters still further, and rendering highly probable the view that the hormone has, on this diet, exerted a beneficial effect on intrauterine growth of the embryos.

Litter size. The low-sodium-high-potassium diet yielded a mean litter

size of 5.25 young as compared with 7.00 for the normal diet. Application of the χ^2 test to the distribution indicated that there were 24 chances in 100 that chance might yield the distribution observed. Fisher's test for the significance of the difference of the means showed that it might occur 22 times in 100 if the diet were without effect. While suggestive that the abnormal salt content of the diet may have reduced the mean litter size, the data are not conclusive.

The influence of A.C.H. on both diets was to increase the mean litter size. The differences observed are, however, not statistically reliable. The fact that the direction of the effect is the same as that observed in the fertility study lends some further confidence in the reality of the effect.

Weight of young. The salt content of the diets used has no demonstrable effect. Correction for the difference in pregnancy duration obliterates the small observed differences.

A.C.H. on both diets, gives slightly greater observed mean birth weights as compared with the corresponding controls. After correction for differences in pregnancy duration, the difference is reduced approximately to zero in the animals on normal salt diets, while that on the low-sodium-high-potassium diet is increased to the point where, while not conclusive, it is strongly suggestive of a favorable influence of the hormone.

Discussion. The outstanding result of this study has been the demonstration that, while A.C.H. administration does not significantly increase the litter weight when the salt content of the diet is normal, it does prevent the reduction which occurs when the sodium content is reduced and simultaneously the potassium content increased. The observation of Thorn, Garbutt, Hitchcock and Hartman (1936) that administration of A.C.H. to normal human beings can cause decrease in sodium and increase in potassium excretion indicates that the hormone may compensate for those abnormalities of mineral composition of the diet which we have employed. Hartman, Lewis and Toby (1937) found that, with repeated injection into dogs, the action of A.C.H. on salt excretion gradually disappeared. Nevertheless, these observations appear to us more significant for our problem than those derived from studies of adrenalectomized animals, particularly in view of the present controversy as to the mechanism of the potassium retention seen therein. That actions of A.C.H., other than that on mineral metabolism may be involved in the influence we have observed on the synthesis of protoplasm during embryonic growth, is of course conceivable. A favorable effect of A.C.H. on the growth of young female rats on a diet of apparently normal salt content has been reported by Fitzhugh (1937).

LACTATION. The third series of experiments sought to determine whether A.C.H. administered to lactating rats, on normal and low sodium diets, influenced the rate of growth of standard litters of eight young.

Methods. Adult female rats, mated with normal males, were maintained during pregnancy on our normal salt diet. On the morning following parturition, any young in a litter in excess of eight were removed. If the litter had fewer than eight, sufficient young were transferred from other litters of not more than one day difference in age to bring the number up to eight. Whenever young died, their places were filled from special litters of similar ages, maintained for this purpose. It was rare that there was more than one death, and in over half of the litters no deaths occurred. The experiment on any one litter lasted 20 days, the limit being set by the fact that the young begin to consume their mother's food shortly before that time. The litters were weighed daily with an accuracy of 0.1 gram.

TABLE 3

Mean weight of young of rats receiving Eschatin or control saline

	DOSE	AGES IN DAYS						INCREMENT*
		Birth	1-5	6-10	11-15	16-20	20	
	cc.	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*	*grams*
Normal salt diet:								
Saline	1.1	5.51	7.04	11.39	16.29	20.27	22.01	13.23
Saline	2.2	5.29	7.60	12.88	17.06	20.99	22.94	13.38
Eschatin	1.1	5.25	7.54	12.12	16.59	20.45	22.67	12.92
Eschatin	2.2	5.55	7.63	12.13	16.51	20.48	21.93	12.84
Low salt diet:								
Saline	1.1	5.28	7.02	11.07	15.42	19.20	20.73	12.18
Saline	2.2	5.42	6.96	10.00	14.37	18.56	19.88	11.60
Eschatin	1.1	5.46	7.76	12.51	16.60	20.59	22.10	12.81
Eschatin	2.2	5.17	6.94	10.85	15.51	19.27	20.86	12.33

* Change from mean of 1 to 5 days to mean of 16 to 20 days.

Forty rats and litters were used, half on our normal salt diet and half on our low sodium diet. On each diet, five rats received 1.1 cc. Eschatin intraperitoneally daily, five received 2.2 cc. Eschatin, five 1.1 cc. 0.5 per cent phenol and five 2.2 cc. 0.5 per cent phenol. Gaunt and Tobin (1936) have found that 2 cc. Eschatin daily restored normal lactating capacity to adrenalectomized rats. Our dose of 2.2 cc. should be adequate, then, to give a supernormal concentration of the hormone in the body tissues even if the adrenal glands should completely cease activity in the presence of hormonal excess. Lest this quantity be toxic, we also used the dose of 1.1 cc.

Results. Effect of sodium content of diet. Table 3 contains the pertinent data. The control litters (those given 1.1 and 2.2 cc. of phenol-saline considered together) on the normal diet show a slightly greater mean

weight increment (13.31 ± 0.60[2] grams) than those on the low sodium diet (11.92 ± 1.02 grams). The difference might occur once in four times if the diet were without effect. While not entirely reliable, this finding suggests that the reduction of the sodium content of the diet from 0.37 per cent to 0.07 per cent somewhat impaired the lactation capacity of the animals.

Effect of A.C.H. On the normal salt diet the mean weight increment of the A.C.H. litters was slightly smaller (12.88 ± 0.45 grams) than that of the controls (13.31 ± 0.60 grams); on the low sodium diet, on the other hand, the reverse is true (controls, 11.92 ± 1.02 grams; A.C.H., 12.57 ± 0.75 grams). None of the differences are statistically reliable. The fact that they are in the direction which would be expected from the sodium-conserving action of the hormone, and thus align themselves with the differences observed in the pregnancy study, lends some credibility. Such differences, however, are far from demonstrating any clear-cut influence of the hormone on lactation capacity. It must be admitted that a diet still lower in sodium content, which would have reduced lactation capacity more distinctly, would have provided more suitable conditions for a demonstration of the effect of the hormone, which our findings suggest exists.

General discussion. In this survey of the effects of adrenal cortical hormone preparations given at various phases of reproductive activity, the direction of effect has been consistently one of increasing the level of activity, at least when the mineral metabolism-regulating function of the hormone has an opportunity to make its influence felt. It may be significant that the clearest results were obtained in the pregnancy study, 1, in which the dosage (0.8 rat unit per day) was the largest used (0.32 rat unit per day in that on fertility and 0.18 and 0.35 rat unit per day in that on lactation); 2, in which the A.C.H. preparation was free from the possible complicating effects of the phenol preservative; and 3, in which the increased potassium content of the diet gave the hormone an opportunity to benefit the organism by improving the excretion of this toxic element.

The bearing of our results on the secretory activity of the adrenal during pregnancy and lactation may finally be considered. Gaunt and Tobin (1936) found that "extract sufficient only to keep an adrenalectomized mother in fairly normal condition will not be sufficient to support a normal lactation. The fluid and electrolyte drains involved in the secretion of milk require, we presume, an extra amount of hormone." But, in spite of the well-recognized tendency of the adrenal to hypertrophy in response to increased demand for its hormone, no such hypertrophy occurs in lactation in the rat (Donaldson, 1924), as it apparently does occur in the guinea pig according to Castaldi (1923). Nor does adrenal hypertrophy occur

[2] The number following the ± sign represents the standard error of the mean.

in pregnancy, according to Donaldson. These findings suggest that the normal-sized adrenal gland of the female rat secretes a quantity of cortical hormone adequate both for maintenance and reproductive activity, provided that the dietary mineral content be favorable. Under these conditions, as we have shown, administration of excess hormone is without beneficial effect. However, if the additional factor of an unfavorable mineral content in the diet be imposed, the physiological secretion rate of hormone is inadequate to cover all needs and the reproductive activities suffer. Under these conditions, as we have shown, provision of additional quantities of the hormone removes the deficiency and permits normal reproductive performance.

SUMMARY

1. Adrenal cortical hormone (A.C.H.) given to normal sexually-mature rats before mating has a marked tendency to increase the incidence of pregnancy and the number of fetuses. This action is exerted on the female to a much greater degree than on the male.

2. A.C.H. given to females throughout pregnancy, when on a diet containing normal quantities of sodium and potassium, has no effect on the duration of gestation, on total litter weight or mean weight of the young, but may slightly increase the number of young delivered.

3. A diet below normal in sodium and higher in potassium given to pregnant control animals has some tendency to reduce total litter weight by reducing the number of young. It has no definite effect on pregnancy duration nor on mean weight of the young.

4. On this low-sodium-high-potassium diet, A.C.H. given through pregnancy definitely increases the mean total litter weight, both by increasing the number of young delivered and their mean weight, over the corresponding values for controls on the same diet.

5. The lactation capacity of the normal albino rat feeding eight young is probably somewhat impaired by reduction of the sodium content of the diet from 0.37 per cent to 0.07 per cent. On the normal sodium diet, A.C.H. may slightly reduce, on the low sodium diet slightly increase, lactation capacity.

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LOCALIZED RESPONSE OF THE MUSCLE FIBER TO EXCITATION TRAVERSING A QUIESCENT AREA

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The fully conducted response, characteristic of the skeletal muscle fiber, may give place under certain limited conditions to a localized effect subject to gradation; and a recent analysis of these conditions (Steiman, 1937) has made it possible to determine selectively the occurrence of one or the other of the two types of contraction. The localized contraction was frequently seen to be separated by a quiescent interval from the site of stimulation. This "distant" or ectopic response appeared most often after injury or contracture of the fiber, and always within the restricted radius (about 1 mm.) shown by Gelfan and Gerard (1930) to limit a graded effect. Intervals of distance, however, within the field thus limited may admit of as much as 1000 microelectrode diameters.

The ectopic response is of lower threshold than that obtainable at the site of stimulation, and can be elicited by either the unipolar or the bipolar method. The quiescent interval may be devoid even of the slightest traction from the active area. Increasing intensity of stimuli will cause progressive extension of the active toward the stimulated area—an effect like that observed by Hou and Brücke (1930) in the stem of Vorticella, and by du Buy (1936) in holothurian muscle (Thyone).

In the following experiments two alternative explanations have been considered: spread of the stimulus itself through electrically conducting media; and physiological excitatory transmission, as suggested by initial results (Steiman and Pratt, 1936).

METHOD. The transilluminated membrane preparation of the tongue (Pratt and Reid, 1930) was used with the leopard frog, R. pipiens, as previously described (Steiman, 1937). Controlled condenser discharges were led through microelectrodes (0.25–4.0 μ diameter, drawn in quartz) applied each in conjunction with an indifferent terminal, or less often in pair with a second stigmatic electrode. The specific need for direct and rapid shift of stimulation from point to point in the low-power field of the compound microscope was met by one or more units of the manipulator devised by J. H. Emerson (Chambers and Kopac, 1937, p. 67), with which

it was possible by movement of a single lever to produce exactly comparable movement of a mounted electrode.

EXPERIMENTS AND RESULTS. *Change in intensity of stimulus.* A fiber was given a stimulus sufficient in strength to evoke a response that included the continuous area immediately above and below the point of application. On decrease of the stimulus, contraction ceased beneath the electrode; but a contraction in part of the originally active area appeared at a distance on one side. If such a result were from direct stimulation of the sarcomeres an effective stimulus should continue to produce a response in the region immediately beneath the electrode, since the current density is greatest at this point (Steiman, 1937). But the distant response, obtained actually with *diminished* stimulus, takes place where current density is less than that necessary to produce a contraction at the point of stimulation. In view of this it is improbable that the ectopic effect can result from direct spread of the stimulus.

Change in position of active electrode. A related possibility is that in unipolar stimulation the lines of current flow between active and indifferent electrode are favorable for stimulation of some other, more irritable portion of the fiber. If this were so, then on shifting the position of the indifferent electrode a change in response might be expected, since the lines of current flow would be altered. At some position of the indifferent electrode the distant response might even disappear, since the more excitable region would not be stimulated. The indifferent electrode was therefore placed successively in various parts of the immersing fluid (Ringer), on the body of the frog, and at the base of the tongue. The response persisted regardless of position or current-direction.

The possibility of surface-membrane conduction. Again on the assumption that the ectopic contraction is a result of electrical conduction, the surface membrane may be conceived to act as the physical conductor. A stimulus producing the distant effect, being freely transmitted by the membrane, would be acting upon the most excitable region of the fiber. Hence, a stimulus of like intensity applied at any point whatever on the fiber should produce a response in this more irritable area. The electrode was accordingly placed at E, figure 1, and a response obtained at C. If the surface membrane were functioning as a physical conductor, then placing the electrode at E_1, E_2, or E_3 (this being a branched fiber) should also produce a response at C (assuming C to be the more excitable area). This did not occur; for when the electrode was placed at E_1 there was no response at C. Instead, distant contraction could be obtained in another region of the fiber, C_1. With the electrode at E_1, and repeating with the same strength of stimulus, there might now be no response. If thereupon the stimulus was increased to threshold for the fiber at that point the response was usually maximal. But after a few such stimuli at E_1 the in-

tensity could again be reduced and the ectopic contraction obtained at C_1. A similar result followed with the electrode at E_2 or E_3: responses were obtainable at C_2 and C_3, respectively, but never at the apparently accessible area C.

Objection can be raised that each shift of the active electrode must alter the electric field, and with it the exciting potentials assumed in the test to be uniform. It will be recalled, however, that changes in the position of the indifferent electrode proved of no significance. In order to determine the possible effect of changing the lines of current flow by shifting the active electrode, this was moved about to see if the response at C, figure 1, could be obtained with the electrode at any other point whatever than at E. In the course of such exploration, points must be encountered of uniform as well as of possibly diverse potential. Assuming a—b to describe an equipotential line, the intensity of the stimulating current should be the same with the electrode at any point on a—b. Since this equipotential line was undetermined, the electrode was actually placed at various points—not only in and about the course of the assumed line, but throughout the whole region—the stimulus being kept at uniform intensity. With the electrode at E_4, for example, there was no response at C. Since the threshold value of a given stimulus varies with the angle of the lines of current flow (Rushton, 1930), the potential had here to be increased to produce a response. But a threshold stimulus for the fiber as a whole (all-or-none effect) still failed to produce afterward a localized response at C. An effective stimulus applied at E_4 would usually cause first this maximal response. After a few responses, however, the potential could be decreased and a localized response obtained in some other region (at the point C_1, for example), but never at C.

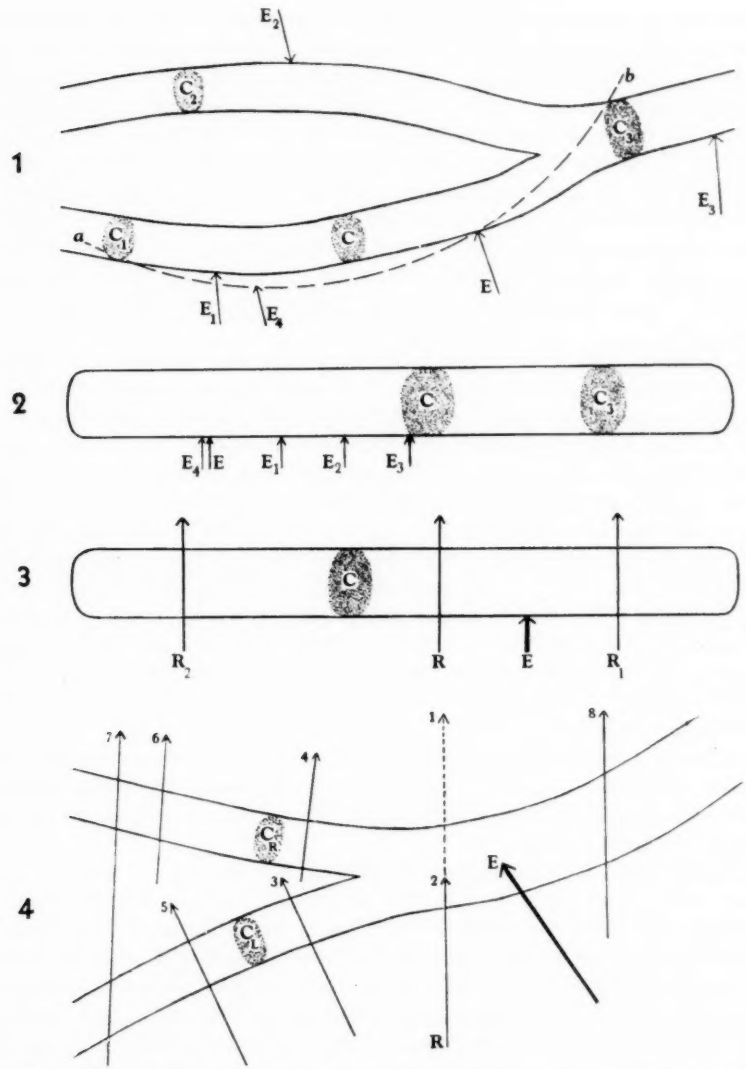
Again, with the active electrode placed at any given point on the fiber or in the immersing fluid other than at E, figure 1, the position of the *indifferent* electrode was continuously changed in attempts to gain the same electric field that existed with the active electrode placed at E. But none of these attempts resulted in a localized contraction at C. It was further assumed that the lines of current flow passing through C, with the electrode at E, have their equivalent when the electrode is placed at E_1, which is the same distance from C as is E. These two sets of current lines would be symmetrical, so that with the electrode at E_1, C should receive the same stimulus that it had when the electrode was at E; the result being presumptively a localized contraction at C. The effect of the electrode at E_1, however, has been described; that is, a response was obtained at C_1 but not at C.

The same results were obtained with two stigmatic electrodes. With these applied at E, figure 1, a localized contraction occurred at C. Keeping the distance between the two electrodes constant in order to maintain the

same electric field, the electrodes were then placed at E_1. There was no response at C, but one could now obtain a contraction at C_1. If the electrodes placed at E were now moved slightly from the fiber, the same stimulus was ineffective; but upon increasing the stimulus the ectopic response at C could be regained. Likewise, if the electrodes at E_1 were removed from the fiber, a small increase in the stimulus would produce a response at C_1, but not at C.

Although stimulation at points as indicated above alters the site of response, shift of stimulation along the fiber, as from point E, figure 1, toward the responding area C, fails to do so. Figure 2 illustrates such an experiment. With the electrode at E, figure 2, a response was obtained at C. But with the electrode at E_4 there was no response at C with the same strength of stimulus. However, if it was placed at any point between E and E_3, as at E_1 or E_2, a like stimulus still produced the response at C. Yet if the electrode was placed at E_3 the same stimulus elicited no response; and when it was increased to liminal value the resulting contraction was usually maximal. Upon continued stimulation the threshold fell, and a localized contraction could now be obtained which involved the area from C to C_3 and included the region C. Still further stimulation caused an added fall in threshold, and by reducing the stimulus one could now obtain, with the electrode at E_3, a contraction at C_3. It appears then that the points E and E_3 mark the limits of electrode position within which the response can be obtained at C—a fact difficult to reconcile with the freedom of spread proper to strictly physical transmission.

Blocking the transmitted effect. If the ectopic response were due to electrical conduction, compression of the quiescent area between the electrode and the active region should have no effect on the response, since this would not interfere with such conduction to a more excitable portion of the fiber. With the electrode at E, figure 3, a contraction was obtained at C. The fiber was uniformly stimulated at regular intervals. A fine glass "rod", R, mounted on a separate manipulator unit, was now applied to the quiescent region; so that by lowering the rod the fiber could be transversely compressed. Very little pressure was sufficient to eliminate the response at C; and when the rod was raised slightly the response returned. It was felt, however, that the lowering of the rod might have separated the fiber from the electrode so as to make the stimulus ineffective. To check this possibility the electrode was lowered, after the fiber had been compressed, until it unquestionably touched the surface. Still there was no response at C. It was only when the block was raised that the contraction reappeared. Moreover, compression of the fiber at any other point outside the region between electrode and responding area (with the rod in position R_1 or R_2, fig. 3) was without effect; for the response persisted. Since the contraction could be eliminated only when



Figs. 1 to 4. The diagrams indicate positions relative to the muscle fiber of microelectrode, E; area of contraction, C, and blocking rod, R. Described in the text.

the region between electrode and area of response was compressed, it seemed that the effect was not from displacement of the fiber: the position of the electrode was not changed when the rod was applied at R_1 and R_2,

and yet the response still appeared at C. Furthermore, since a slight pressure anywhere in this effective region was sufficient to eliminate the response, it is unlikely that the fiber was displaced to an extent great enough to account for the elimination. The fact that the response did not return when the electrode was depressed bears out this contention. Nevertheless, in all cases where the rod was applied outside of the effective region an attempt was made to determine whether the response could be eliminated by compressing the fiber as much as possible. When this was done the response persisted until so much pressure was applied that the fiber was visibly displaced from the electrode; but if the electrode was now brought closer, the same stimulus was again effective in eliciting contraction at C.

Use was now made of a branched fiber. With the electrode at E, figure 4, two ectopic contractions, C_L and C_R were simultaneously obtained, each on a separate branch. Placing of the blocking rod at position R—*1*, so that the entire breadth of the quiescent trunk-fiber was compressed, eliminated both contractions. The rod was then drawn slowly across the fiber until in position R—2. *At this point the response at C_R reappeared.* If the rod was now slightly raised, the contraction at C_L also reappeared. Placing the rod at *3* eliminated the response at C_L, but not at C_R; whereas with the rod in position *4* the response at C_R was eliminated, while that at C_L persisted. But compression at positions *5*, *6*, *7* or *8* proved powerless to affect the responses at C_L and C_R. Hence, although the electric field and the stimulus were throughout unaltered, the response could be abolished only when the fiber was compressed in the region between electrode and active area.

Since the transmitted effect can be blocked by light compression and released, it is not to be ascribed to electrical conduction. The results agree, therefore, in indicating the process as one of excitatory transmission over a region mechanically at rest.

Discussion. The possibility of isolating the conductive process has been much debated. The work and diverse conclusions of earlier investigators have been summarized by Fulton (1925), whose own results strongly favor the inseparability of the basic properties of muscle. It is evident, however, that if a response localized beneath the cathode signifies the excitation of immediate contractile units (Gelfan and Gerard, 1930), the ectopic contraction may involve similar units activated at a distance. But in the light of present results such a system is analogous to the nerve-muscle, where the conductor is of lower threshold than the effector. For in both systems a stimulus made sub-threshold for the direct response may yet remain effective for the conductive process. In the ectopic response this conductive process would admit of interpretation as a separable physiological event.

SUMMARY

1. In the retrolingual membrane of the frog, under conditions favorable for the localized response of a muscle fiber, contraction was observed to occur in the fiber at a distance in the microscopic field from the site of stimulation.

2. It was determined that electrical conduction of the stimulus through electrolyte or surface membrane was not accountable for this ectopic response.

3. On the other hand, the transmitted effect could be mechanically blocked by transverse compression of the quiescent region separating electrode and area of response. The process was freely reversible.

4. Excitatory transmission can thus apparently take place in a non-contracting interval, indicating the separability within these muscle fibers of the functions of conduction and contraction.

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THE QUESTION OF CARDIAC HYPERTROPHY DURING PREGNANCY

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There is still a divergence of opinion as to whether or not pregnancy produces cardiac hypertrophy. The late Dr. J. W. Williams in his widely used textbook of obstetrics (1) definitely stated that pregnancy caused cardiac hypertrophy in human beings. Stander and his co-workers (2,3), however, are not certain whether pregnancy actually produces a cardiac hypertrophy, although they stress the fact that pregnancy throws an additional load on the heart. White (4) specifically states that if there is a cardiac hypertrophy during pregnancy it is very slight.

In view of the difference of opinion on this subject it was thought worth while to make an investigation of the matter. The problem not only is of interest from the standpoint of the heart in pregnancy, but also from the standpoint of the mechanism of cardiac hypertrophy.

METHODS. Healthy young pregnant guinea pigs were used in this work. They were fed an adequate diet and were kept in a clean suitable environment. One group was allowed to have its young and within 72 hours after parturition each mother was weighed and then killed by a blow on the head. The thorax was opened and the heart removed from the pericardial sac. The great vessels were cut flush with the surface of the heart; the chambers of the heart were slit open and washed free of blood. The excess moisture was removed from the organ by blotting it with filter paper. The heart was then carefully weighed. The data are expressed as the amount of heart substance for each kilogram of body weight.

In another group of animals pregnancy was allowed to proceed for about 7 weeks. They were then killed and the hearts removed and weighed as previously described.

Some data were also obtained from 10 cats and 7 dogs which were well advanced in pregnancy. The technique used for killing these animals and weighing their hearts was essentially the same as that used in obtaining the data from the guinea pigs.

RESULTS. Table 1 shows the data obtained from the guinea pigs. It will be noted that pregnancy did not cause a cardiac hypertrophy. The animals which were killed before the young were born showed a smaller

HW/BW ratio than did the control animals. However, after the weight of the uterine contents had been subtracted from the body weight, the heart showed exactly the same proportion to the body weight as it did in the controls.

The animals which were allowed to have their young and then sacrificed likewise showed no cardiac hypertrophy.

Table 2 is designed to show that variations in body weight are of little significance as regards the HW/BW ratio. In this table the HW/BW ratios in normal animals are presented by weight groups. The data show

TABLE 1

Effect of pregnancy on the HW/BW ratio in the guinea pig

NUMBER OF ANIMALS	CONDITION	AVERAGE BODY WEIGHT	AVERAGE HW/BW RATIO
		grams	*grams per kilo*
90	Normal	429	3.17 ± 0.03
26	Antepartum	679	2.93 ± 0.05
26	Antepartum corrected*	617	3.17 ± 0.04
27	Postpartum	648	3.06 ± 0.05

* Weight of uterine contents subtracted from body weight.

TABLE 2

HW/BW ratios by weight groups in the guinea pig

BODY WEIGHT GROUP	AVERAGE BODY WEIGHT	HW/BW RATIO
grams	*grams*	*grams per kilo*
200–350	288	3.26 ± 0.05
351–450	407	3.22 ± 0.07
451–550	498	3.00 ± 0.04
551–650	595	3.17 ± 0.06
651–	697	3.06 ± 0.08
Average	429	3.17 ± 0.03

that there is a slight tendency for this ratio to decline in the heavier animals. It is of little significance, however, since the probable errors of the various means cause them to overlap the mean of the whole series in all except one case—in the middle of the range (451–550 grams). The heart in normal animals in this series, for all practical purposes, gains in weight as the body does.

The data in table 3 show the results obtained from 10 cats and 7 dogs. While these data are too meager to be conclusive, they nevertheless corroborate the results obtained from the guinea pig.

DISCUSSION. As previously mentioned, it has been shown by Stander

and his co-workers (2,3), that cardiac output is greatly increased during pregnancy. A number of other workers have made the same observation. The data set forth in this paper, however, show that pregnancy did not produce any demonstrable cardiac hypertrophy. This may be taken as additional evidence that increased cardiac work does not necessarily lead to hypertrophy. It is known, too, that pregnancy is associated with hypervolemia. It would not be unreasonable to suppose that the hypervolemia would cause a cardiac dilatation with a subsequent hypertrophy. In spite of the hypervolemia, however, the heart did not increase in weight during pregnancy.

The physiologists and clinicians who believe that cardiac hypertrophy cannot be caused by increasing the amount of work the heart has to do, would not expect a normal pregnancy to produce a cardiac hypertrophy. These men believe that cardiac hypertrophy is a response to some former

TABLE 3

Effect of pregnancy on the HW/BW ratio in the cat and dog

ANIMAL	NUMBER OF ANIMALS	CONDITION	AVERAGE BODY WEIGHT	AVERAGE HW/BW RATIO
			grams	*grams per kilo*
Cat	90	Normal	1,888	3.94
	10	Antepartum	2,072	3.72
	10	Antepartum corrected*	1,909	4.02
Dog	47	Normal	8,310	7.66
	7	Antepartum	7,697	6.90
	7	Antepartum corrected*	7,159	7.31

* Weight of uterine contents subtracted from body weight.

injury of the heart muscle. The evidence presented in this paper would favor the view of this group, namely, that increased work does not necessarily produce cardiac hypertrophy.

The fact that no demonstrable cardiac hypertrophy was found in the guinea pig, the cat and the dog, during pregnancy suggests that probably none occurs in the human either. While obvious objections can be raised to applying this to humans, it appears to the authors that the burden of proof lies with those who claim that the data are not applicable. Most of the data obtained from human beings are open to criticism. It is difficult, if not impossible, to judge the heart size in the human being during pregnancy. The enlarged uterus displaces the heart upward and the enlarged breasts too make it more difficult to see the heart shadow. Evidence obtained by the x-ray, therefore, should be accepted with a great deal of caution. Most of the data, moreover, published on humans have

been quite inadequate and have not lent themselves to a statistical analysis. Furthermore, figures obtained from human autopsy material could scarcely be considered normal figures. In fact it is hard to see how this problem definitely can be decided in the case of the human subject.

Another factor which needs comment is the period of gestation. In the guinea pig, as in the cat and dog, the period of gestation is about 63 days; in man it is about 280 days. The criticism may be made that due to the great difference in the duration of the gestation periods between the animals mentioned and man, comparisons should not be drawn. This criticism may be answered by the fact that the smaller animals have a much shorter span of life than does man. Furthermore, the increased weight of the uterus in these animals during pregnancy is much greater in proportion to the body weight than in the case of the human subject; this should favor cardiac hypertrophy in the smaller animal. It, too, must be stated that nine weeks is more than ample time to produce cardiac hypertrophy; experimentally it has been shown by Eyster (5), Van Liere (6) and others that it may be produced in considerably less time.

SUMMARY AND CONCLUSIONS

The normal HW/BW ratio in 90 normal adult female guinea pigs was found to be 3.17 grams per kilo. Twenty-six animals were killed within 72 hours after they had given birth to their young; the HW/BW ratio in these animals was found to be 3.06. Twenty-seven pregnant animals were killed during the latter part of pregnancy and the HW/BW ratio was found to be 2.93. After the weight of the uterine contents had been subtracted from the body weight, however, the HW/BW ratio was found to be 3.17, that is exactly the same as in the control animals. Corroborative data were also obtained from 10 cats and 7 dogs.

The conclusions drawn from this work are: 1. Pregnancy does not cause cardiac hypertrophy in the guinea pig. (Nor was there any evidence of cardiac hypertrophy in 10 pregnant cats and 7 pregnant dogs.) 2. Since pregnancy does not produce cardiac hypertrophy in 3 different types of animals it seems doubtful that it would produce it in human beings. 3. Increased cardiac work does not necessarily produce cardiac hypertrophy.

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THE BLOOD FLOW AND OXYGEN CONSUMPTION OF THE KIDNEY IN EXPERIMENTAL RENAL HYPERTENSION[1]

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Goldblatt and his associates (1934) have shown that hypertension may be produced with great regularity by constricting the renal arteries. Ingenious silver clamps were devised for this purpose. The etiology of the hypertension so produced has not yet been elucidated. However, evidence has been summarized elsewhere (Goldblatt, 1937; Blalock and Levy, 1937) which indicates that the mechanism is hormonal rather than nervous. The production of the hypertension by this method has been assumed to depend on renal ischemia. It has been shown that interference with the blood supply of one or both kidneys by constricting or ligating the renal arteries, by obstructing the ureters or by dividing the pedicle completely except for vein and ureter results in a rise in blood pressure (Blalock and Levy, 1937). The present studies deal with the effects on the renal blood flow and oxygen consumption of constriction of the renal arteries with resulting hypertension. Further, the blood pressure in the renal artery distal to the point of constriction has been determined.

METHOD. Hypertension was produced in dogs by constricting the renal arteries by means of silver clamps (cf. Goldblatt and associates, 1934).[3] The blood pressure was determined by direct puncture of the femoral artery. The renal blood flow was measured approximately one week before and once or twice at varying intervals after a well marked, sustained hypertension had developed. When necessary, the clamps were tightened in order to maintain the elevation in blood pressure. The blood flow was determined without anesthesia by the use of the cannula devised by Mason, Blalock and Harrison (1937). The animals were trained to lie quietly on the table and a small amount of novocaine was injected at the site of insertion of the cannula. The dogs exhibited no signs of discomfort. The rate of flow from the renal veins was measured and a sample of renal vein blood was taken from the rubber tube connected to the cannula. Simultaneously, a sample of blood was withdrawn from the femoral artery.

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[2] National Research Fellow in the Medical Sciences.

[3] Clamps furnished by courtesy of Doctor Goldblatt.

The oxygen content of the blood samples was determined by the Van Slyke-Neill technic.

After the final measurement of the blood flow, some of the animals were anesthetized with ether, the abdomen opened and the blood pressure in the renal arteries distal to the clamp determined by inserting into the lumen a needle connected to a mercury manometer. In two dogs not included in the blood flow group, the blood pressure in the renal arteries distal to the clamp was determined under local anesthesia, the kidneys

TABLE 1

The effects of constriction of the renal arteries on the blood flow and oxygen consumption of the kidneys

DOG NUMBER	WEIGHT	CONTROLS						AFTER CONSTRICTION						
		Blood pressure	Kidney flow	Blood O_2		Average difference	Renal O_2 consumption	Days after clamps applied	Blood pressure	Kidney flow	Blood O_2		Average difference	Renal O_2 consumption
				Systemic artery	Renal vein						Systemic artery	Renal vein		
	kgm.	*mm. Hg*	*cc. per minute*	*vol. per cent*	*vol. per cent*	*vol. per cent*	*cc. per minute*		*mm. Hg*	*cc. per minute*	*vol. per cent*	*vol. per cent*	*vol. per cent*	*cc. per minute*
1	9.85	135	227	15.98	13.13	2.85	6.47	57	190	136	14.87	12.73	2.14	2.91
2	12.0	130	230	15.69	13.08	2.61	6.00	15	192	123	13.95	10.68	3.27	4.02
3	14.6	134	454	17.89	15.53	2.36	10.71	9	180	283	17.31	14.97	2.34	6.62
4	14.0	138	312	24.09	21.98	2.11	6.58	11	175	205	21.61	19.37	2.24	4.59
5	11.0	140	178	17.39	14.27	3.12	5.55	19	182	146	16.23	14.65	1.58	2.31
6	14.4	125	288	19.28	17.13	2.15	6.19	14	180	154	18.07	16.16	1.91	2.94
7	9.75	138	306	17.38	12.93	4.45	13.62	12	175	172	18.27	13.42	4.85	8.34
8	13.8	145	348	14.58	12.07	2.51	8.73	13	195	186	15.28	12.80	2.48	4.61
9	12.8	130	273	15.49	12.07	3.42	9.34	6	180	102	14.64	10.37	4.27	4.36
10	13.9	115	294	19.62	16.17	3.45	10.14	73	176	208	14.53	11.47	3.06	6.36
Aver....	12.6	133	290.6	17.74	14.84	2.90	8.33	21	181	171.5	16.44	13.57	2.814	4.71

having been explanted under the skin at a previous operation. For comparison, the blood pressure in the femoral artery was determined also.

Results. The results of the determinations of the renal blood flow, arteriovenous oxygen difference and oxygen consumption are given in table 1. There was a marked reduction in the renal blood flow after constriction of the renal arteries had been present for from 6 to 73 days, averaging 41 per cent of the control flow. The arteriovenous oxygen difference, however, remained approximately the same confirming the observations of Mason, Evers and Blalock (1937). In consequence, the oxygen consumption was lessened in approximately the same proportion as the reduction in blood flow (43 per cent).

The average blood flow per gram of kidney tissue following constriction of the renal arteries was 2.7 cc. per gram per minute. The control flow per gram of kidney was determined in several ways. Kidney weights were calculated from the body surface area using the factors 96.2 × square meters (Van Slyke et al., 1934) and 102.0 × square meters (Stewart, 1921). The surface area was calculated by the weight-length formula of Cowgill and Drabkin (1927). In addition the weights of kidneys of normal dogs as found in the literature were used for comparison (Blalock, 1934; Stewart, 1921). In the latter, only the data concerning dogs the body weights of which were approximately the same as the average in the present studies were used and the estimated kidney weight was computed from the kidney weight-body weight ratio. The average estimated normal kidney weight for 12.7 kgm. dogs ranged from 61.7 to 67.7 grams (average weight after hypertension was produced was 63.1) omitting the highest estimated value of 82.35 grams (computed from normal kidney weight-body weight ratios obtained from Stewart, 1921). Using this latter figure, which was 14.7 grams greater than the next highest estimate, the average blood flow per gram of kidney tissue in the control studies is 3.5 cc. per gram, indicating that a decreased blood flow per gram kidney is present following constriction of the renal arteries. Furthermore, the normal blood flow per gram of kidney tissue determined by Van Slyke and his associates (1934) in 18 studies on 11 dogs is 4.01 cc. per gram of kidney tissue as compared with 2.7 cc. per gram in our studies on dogs with hypertension. The total renal blood flow determined by Van Slyke averaged 284 cc. per minute in dogs with an average weight of 13.6 kgm. In our control determinations the total renal blood flow averaged 291 cc. per minute in dogs with an average weight of 12.7 kgm.

The blood pressure in one or both renal arteries distal to the clamp was determined in eleven instances. Normally, the blood pressure in the renal arteries is slightly above that in the femorals. In every case, the blood pressure in the renal arteries was definitely below the level of the corresponding femoral artery pressure, averaging 50 mm. and 38 mm. Hg lower in the right and left renal arteries respectively.

Two of the eleven determinations were performed under local anesthesia. The findings in these animals were similar to the determinations made under ether anesthesia.

Due to the fact that occasionally the clamps had to be tightened in order to maintain the elevation in the blood pressure, the number of days after the clamps were applied as listed in the table does not necessarily indicate the number of days hypertension was present. Several days were usually required for the maximum rise in blood pressure to be reached.

The renal blood flow and oxygen consumption were determined a second time after constricting the renal arteries in five instances. These experi-

ments gave the same results as the previous determinations and are therefore not included in the table. It was noted in the three animals in which the clamps were not adjusted between the second and third flows, that there was a slight increase in the blood flow and a slight decrease in the systemic blood pressure. This is probably due to improvement in the collateral circulation of the kidneys.

Discussion. As stated above, the mechanism of the production of experimental hypertension by constriction of the renal arteries is not known but renal ischemia has been assumed to be of fundamental importance in initiating the rise in blood pressure. That renal ischemia is present has been shown by the present studies. There is a decrease in blood pressure in the renal arteries as well as a decrease in blood flow through the kidneys. Anoxemia in consequence of the decreased blood flow has not been demonstrated, however. The arteriovenous oxygen difference remains approximately the same in spite of a decreased flow of blood through the kidney.

The normal renal arteriovenous oxygen difference is usually quite small (cf. Mason, Blalock and Harrison, 1937). Blalock and Bradburn (1930) in barbitalized dogs showed that the renal arteriovenous oxygen difference is usually not markedly increased by hemorrhage, injection of histamine, cerebral trauma, etc., while the arteriovenous oxygen differences in other parts of the body are usually greatly increased.

An elevation in blood pressure as a consequence of ureteral occlusion has been reported by a number of observers. Levy, Mason, Harrison and Blalock (1937) determined the blood flow and oxygen consumption of the kidney in acute hydronephrosis, three of eight dogs studied developing hypertension after obstruction of the ureters. An average reduction in the blood flow of 41 per cent was found. The average reduction in the blood flow in the present studies was also 41 per cent. The arteriovenous oxygen difference was not increased following ureteral obstruction. These results indicate that hypertension produced by constriction of the renal artery and by obstruction of the ureter probably have essentially the same origin.

Dock and Rytand (1937) in a study in rats of the renal blood flow after subtotal nephrectomy found that the rate of flow per gram of kidney tissue was not reduced although the total blood flow was less than in the controls. They conclude that "rats which become hypertensive several months after subtotal nephrectomy do not have renal ischemia." In our experiments, the evidence indicates that constriction of the renal arteries is associated with a reduction in the blood flow per gram of kidney tissue as well as in the total renal flow.

SUMMARY

The blood flow and oxygen consumption of the kidneys were determined before and after producing hypertension by constricting the renal arteries.

The blood pressure in the renal arteries distal to the clamps was determined after hypertension had developed. The blood flow and oxygen consumption were found to be reduced, the arteriovenous oxygen difference remaining practically unchanged. The blood pressure in the renal arteries distal to the constricting clamp was reduced in all determinations.

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THE LIVER LIPIDS AND FECAL EXCRETION OF FAT AND NITROGEN IN DOGS WITH LIGATED PANCREATIC DUCTS

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In 1936 Prohaska, Dragstedt and Harms (1) stated that fatty degeneration and infiltration of the liver occurring in depancreatized dogs is not due to the absence of pancreatic juice, as it does not occur in dogs with total pancreatic fistulae or ligated pancreatic ducts. Of the seven fistula animals observed, three exhibited, after 3 to 5 weeks, slight fatty infiltration of the liver which was ascribed to intercurrent infections. In the remaining four, which were sacrificed after 4 to 6 weeks, the liver was found to be normal. Three dogs with ligated pancreatic ducts died in 6, 8 and 12 weeks; sections of the liver showed slight fatty infiltration in two. These results are of significance as part of the proof of the presence of a fat-metabolizing hormone, lipocaic, which is believed by Dragstedt et al. (2) to be elaborated as an internal secretion of the pancreas and to be the factor in raw pancreas which prevents fatty livers.

The results of Aubertin et al. (3), of Loewy (4), and of Berg and Zucker (5) do not agree with these findings. Aubertin (3) observed fatty livers in 3 dogs whose pancreatic ducts had been ligated for 4, 8 and 20 weeks. In one fistula dog observed by Loewy and 10 by Berg and Zucker, fatty livers were noted when the animals died after 3 to 8 weeks.

Because of the contradictory nature of these reports, and in view of the fact that the conclusions of all these investigators were based on gross and histological examinations of the liver, and are not supported by chemical analyses, we are reporting the effect of complete ligation of the pancreatic ducts on the total liver lipids and their fractions in three dogs. In addition, the excretion of fat and nitrogen in the feces of these duct-ligated dogs was also studied.

In three adult female dogs the pancreas was separated completely from the duodenum, the ducts were ligated distally and proximally and then cut and a piece of the omentum was inserted between the separated pancreas and the intestine in order to provide against the reëstablishment of any pancreatic sinuses. The completeness of ligation was checked when the animals were sacrificed.

The basal diet and care of these dogs, as well as the methods used for liver analysis, have previously been described by us (6). In addition to the basal diet, these animals received the following daily supplements throughout the experimental period: dog 1, 20 grams mazola (iodine number 118–125) and 10 grams crude egg yolk lecithin (John Carle and Son) (iodine number 110); dog 2, 20 grams cod liver oil (Mead Johnson) (iodine number 167) and 10 grams lecithin; dog 3, 30 grams crisco (iodine number 70). The diets used consisted of 53–59 per cent carbohydrate, 23 per cent protein (N × 6.25) and 18–24 per cent fat and provided 1100–1300 calories per day. The feces were collected for periods of 2 to 4 days, stored under 95 per cent alcohol and analysed immediately at the end of the collection period. In both food and feces nitrogen was estimated by the Kjeldahl method, lipids by the technique described by Sperry (7).

Frequent examinations of the urine for sugar (qualitative Benedict) were always negative in all 3 animals. Blood sugars done by the Folin-Wu method ranged between 76 and 111 mgm. per 100 ml. At autopsy, the pancreas was greatly reduced in size.

Analysis of a 10 gram sample of liver removed at the time the pancreatic ducts were ligated provided normal control values for the individual animal. After ligation periods of 13, 13.8 and 15 weeks, the dogs were sacrificed and another 10 gram sample of the liver was submitted to lipid analysis. The results are summarized in table 1. The values for the liver lipids during the normal periods fall within the range previously reported for normal dogs on the diets used (6). In all 3 animals at the end of the pathological period, the total liver lipids increased from the normal values of 5.78, 4.65 and 4.46 per cent (of the wet weight of the liver) to 29.9, 16.8 and 12.8 per cent in dogs 1, 2 and 3, respectively. This increase was due almost entirely to an increase in the neutral fat fraction, and is reflected in the decrease of the iodine number of the total fatty acids from values of 131, 147 and 132 to 77, 81 and 76. In all three, there also occurred a decrease in the concentration of phospholipid and an increase in cholesterol esters, while the values for free cholesterol remained within the normal range. These changes parallel closely those observed in the livers of totally depancreatized dogs by Kaplan and Chaikoff (8) and in this laboratory (9).

The values for the percentage absorption of lipids and nitrogen given in table 1 have been calculated from the analyses of food and feces. The average values for a series of 20 normal dogs on similar diets studied in this laboratory were 97.3 ± S. D. 1.9 per cent lipid absorption and 94.5 ± S. D. 2.4 per cent nitrogen absorption. Following ligation, the percentage absorption of lipid and nitrogen fell to average values of 72 per cent and 73 per cent respectively (average of 3 periods) for dog 1, to 72 per cent and 72 per cent (2 periods) for dog 2, and 47 and 33 per cent (2 periods) for dog 3.

The two dogs which absorbed a greater percentage of fat from the diet also exhibited the higher liver lipids values of 29.9 and 16.8 per cent in spite of the fact that they received a daily supplement of lecithin throughout the experimental period. The crude egg yolk lecithin was analyzed for its choline content by the Reineckate method of Beattie (10) and the periodide dilution method of Mann and Quastel (11), and yielded a choline value of 9.6 and 9.9 per cent respectively. The daily average intake of choline from the lecithin fed was therefore 0.98 gram. To this should be added 0.31 gram of choline which was contained in the basal diet. Dogs 1 and 2 therefore received 1.29 gram of choline daily. Unpublished observations from these laboratories indicate that at least 2 grams of choline per day, administered prophylactically to depancreatized dogs, are necessary to prevent fatty infiltration of the liver in dogs fed similar diets. Even this amount is not uniformly effective in preventing some degree of fatty infiltration. Quite possibly the lack of effectiveness of the lecithin in dogs 1 and 2 was due to the lowered choline intake which was not enough to prevent a severe degree of fatty infiltration. As the cholesterol content of the crude lecithin was only 0.30 per cent, it is not likely that this was a factor in causing the fatty infiltration.

Since Channon and Wilkinson (12) have shown that a low protein intake (below 10 per cent) is associated with the production of fatty livers in rats, it seems desirable to consider our results in relation to the amount of protein available to the animals. While the diet provided 23 per cent protein ($N \times 6.25$), this percentage fell to 17, 17 and 8 per cent absorbed in dogs 1, 2 and 3, respectively. Actually, in proportion to the amount of fat absorbed, these percentage values are higher. There is, however, no apparent relation in these dogs between protein absorption and the liver lipids. Dog 3, which absorbed only half as much protein as the other two, showed the least fat in the liver.

At the end of the experimental period, dogs 1 and 2 were in good condition in spite of definite loss of weight (table 1), while dog 3 was weak and emaciated. In view of the findings of various investigators on the effect of starvation on the total fatty acids of the liver (13, 14, 15), this loss of body weight must be taken into consideration. Dible (14, 15) found that in both rats and rabbits the extent of fat infiltration which occurs in the liver in starvation is determined by the quantity of fat available for mobilization in the animal's storage depots. In the later stages of starvation, the liver fat is reduced as a result of the utilization of all available depot fat. These considerations may explain the fact that dog 3 had the lowest total liver lipids, since, of the 3 animals studied, it showed the greatest percentage loss of weight and the poorest absorption of fat and protein after ligation of the pancreatic ducts (table 1).

Kaplan and Chaikoff (16) found that although fatty livers appeared as early as 3.5 weeks after pancreatectomy, the occurrence of fatty livers

was not a constant finding at this time. It required a period of at least 16 weeks to insure a finding of fatty acids in excess of 14 per cent in the livers of completely depancreatized dogs receiving no known lipotropic substance. It would seem, in view of the similarity of the changes in the amounts and character of the liver lipids after duct ligation and after pancreatectomy, that the rate of change should be similar in both instances. The discrepancy between Dragstedt's observations and those here reported may be due to the shorter period of time in which Dragstedt and his co-workers observed their animals, namely, 4 to 12 weeks.

It is apparent from these results that complete ligation of the pancreatic ducts *does lead* to an intense fatty infiltration of the liver in the periods of time reported in this paper. Although Chaikoff and Kaplan (17) have

TABLE 1

Liver analyses and lipids and nitrogen absorption in 3 pancreatic duct-ligated dogs

Dogs 1 and 2 received 10 grams lecithin supplement daily

DOG NO.	PERIOD	BODY WEIGHT	PER CENT LOSS OF BODY WEIGHT	LIGATED PERIOD	LIVER*											ABSORPTION OF		TIME AFTER LIGATION
					Weight	% of body weight	Total lipid	Total fatty acid	Iodine No. of T.F.A.	Phospholipid	Neutral fat	Unsaponifiable	Cholesterol			Lipids	Nitrogen	
													Total	Free	Ester			
		kilos		*wks.*	*gms.*		%	%		%	%	%	%	%	%	%	%	*wks.*
1	Normal	19.3					5.78	3.30	131	3.48	0.98	0.75	0.260	0.200	0.060			
	Ligated															70	74	3
		13.5	30	13.8	660	4.9	29.9	25.9	77	2.32	25.5	0.65	0.285	0.150	0.135	74	80	7.4
																73	66	13
2	Normal	18.3					4.65	2.60	147	2.77	0.74	0.57	0.279	0.205	0.074			
	Ligated															69	72	4.3
		13.0	29	15.3	385	3.0	16.8	14.0	81	1.34	13.8	0.81	0.303	0.191	0.112	75	72	9.5
3	Normal	13.0					4.46	2.68	132	3.14	0.61	0.30	0.235	0.209	0.026			
	Ligated															47	34	2.3
		7.0	46	13.3	212	3.0	12.8	10.5	76	1.59	9.6	0.84	0.552	0.295	0.257	46	32	12.4

* Liver lipids values are given in per cent of original tissue.

criticized the practice of conducting lipid analyses on sections of the liver, rather than the whole liver, there can be little doubt regarding the fatty nature of the livers observed in these experiments. These results are not in agreement with the thesis of Dragstedt (2) that a fat-metabolizing hormone is present as an internal secretion of the pancreas, unless it be supposed that the elaboration of such a hormone was effectively curtailed in our animals by the degeneration of the acinar tissue known to occur after ligation of the pancreatic ducts. Berg and Zucker (5), however, reported fatty livers in all their dogs which had pancreatic fistulae. In view of the variable time element involved, it is quite possible that the fistula dogs observed by Prohaska, Dragstedt and Harms (1) did not survive long enough to develop fatty livers.

SUMMARY AND CONCLUSIONS

Ligation of the pancreatic ducts in 3 dogs for periods of 13 to 15 weeks produced fatty changes in the livers which were indistinguishable from those of depancreatized dogs. These changes are considered in relation to the fecal excretion of fat and protein and the loss of weight of the animals. Inclusion of a lecithin supplement in the diet did not prevent the appearance of fatty livers in 2 of the dogs.

These results do not support the contention that a fat-metabolizing hormone is produced by the pancreas.

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RECRUITMENT OF MUSCULAR ACTIVITY AND THE CENTRAL NEURONE AFTER-DISCHARGE OF HYPERPNEA[1,2]

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The respiratory act is a most variable performance, for no two individuals seem to breathe in exactly the same way. In some individuals expiration may be a purely passive act while in others under presumably the same conditions the expiratory muscles participate in varying number. Added to these miscellaneous combinations of muscle use there is a variety of intensity and of sequence of muscular contractions, all of which contribute to an individuality of breathing. The only item of uniformity seems to be the unfailing contraction of the diaphragm and of the intercartilaginous portion of the internal intercostal muscles during the phase of inspiration (Gesell, 1936). Such extreme variability must eventually find its explanation in a specificity of architectural structure of the central nervous system and an uniqueness of pattern of afferent signals which drive and guide the act. Our general interest in the factors contributing toward the integration of the respiratory act has led us to study the manner in which mechanical energy is recruited to ventilate the lungs during respiratory stress.

METHOD. The procedure was the same as that employed in the studies just mentioned. Action potentials of important inspiratory and expiratory muscles of anesthetized dogs were sampled and recorded to permit a comparison of their changing activity. In figure 1, the activity of the M. transversus abdominis is recorded above and the activity of the Mm. intercostales externi is recorded below. The tracheal pressure records, T.P., establish the beginning of inspiration by a downstroke and the beginning of expiration by an upstroke. The tidal air records, T.A., indicate the changes in pulmonary ventilation, but they exhibit considerable time lag due to the inertia of the system. Respiratory stress and its associated hyperpnea were produced by intravenous injection of M/100 solution of sodium cyanide. These injections, lasting from one to three seconds, produced an increase in ventilation of three- to ten-fold, which

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[2] This study was supported in part by a grant from the Rockefeller Foundation.

was followed by a period of recovery lasting from one-half to three minutes.

RESULTS. *Recruitment of muscular activity.* The manner of recruiting mechanical energy during hyperpnea was extremely variable which indicates that individuality of breathing holds for hyperpnea as it does for eupnea. The results permit of no definite classification for they run from one type into another.

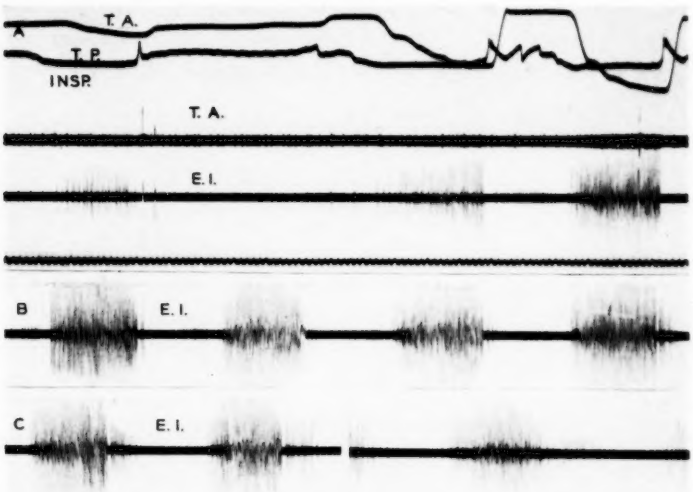

Fig. 1. Hyperpnea in which the extra mechanical energy is supplied exclusively by the inspiratory muscles. *T.A.* and *T.P.* are records of tidal air and tracheal pressure in which downstroke indicates the onset of inspiration and upstroke the onset of expiration. Due to the extensive lag of the spirometer the tidal air record is of value in giving the changes in pulmonary ventilation only. *T.A.*, electrogram of the M. transverse abdominis. *E.I.*, electrogram of the Mm. intercostales externi. Time is indicated in intervals of 1.0, 0.20 and 0.04 second.

Contraction of inspiratory muscles was always augmented during hyperpnea and not infrequently the extra mechanical energy was supplied entirely by the inspiratory muscles, as was the case in figure 1. The Mm. intercostales externi, acting as inspiratory muscles, responded with a marked augmentation of potentials while the M. transversus abdominis showed no activation whatever. Electrical sampling of the remaining inspiratory and expiratory muscles of this individual showed the same increased activity of all of the inspiratory muscles and the same lack of activation of the expiratory muscles during hyperpnea.

In some individuals there was not only augmentation of the inspiratory

contraction, but also a prompt and complete disappearance of the contractions of the expiratory muscles. Hyperpnea thus becomes more inspiratory in character through a simultaneous increase in strength of the inspiratory contractions and a decrease in strength of expiratory contractions.

Occasionally the expiratory muscles were continuously active during eupnea, contracting through both periods of respiration. Hyperpnea under such conditions led to phasic interruption of these contractions with the inhibitions confined to the inspiratory phase. The strength of con-

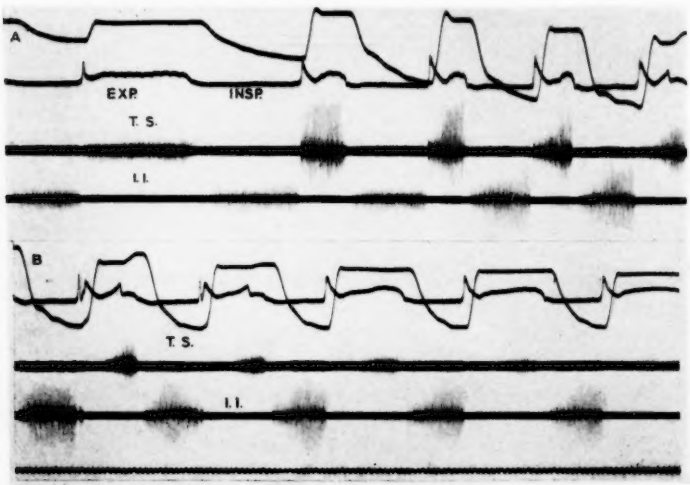

Fig. 2. Hyperpnea in which the expiratory muscles contribute considerable mechanical energy. *T.S.*, electrogram of the M. triangularis sterni. *I.I.*, electrogram of one of the Mm. intercostales interni. Note the rapid decline in strength of contraction of the expiratory muscles and the well maintained activity of the inspiratory muscle.

traction during the expiratory phase remained unaffected. Such inhibitions during the phases of inspirations are probably due to greater irradiations from the respiratory center provoked by the augmented inspiratory discharges. Viewed from the point of mechanics, the sudden relaxation of the expiratory muscles, coming as it does with greater lung inflation, must ease the stretching of these muscles and contribute a decided advantage.

In contrast to these findings, the expiratory muscles often contribute extra mechanical energy towards the conduct of hyperpnea, varying from a slight to a fair proportion. A strong tendency towards early weakening

of the initial augmentation reduces their contributions appreciably. But even with precipitous weakening, the expiratory muscles may play an important part in the early stages of hyperpnea. In figure 2, for example, the M. triangularis sterni begins with a burst of force, but weakens rapidly and ceases to contract before the hyperpnea has transpired. The Mm. intercostales on the other hand, warm up slowly and continue hyperactive throughout the period of respiratory stress. As in figure 1, the results of figure 2 are typical for the remaining respiratory muscles of the individual.

Intermediate effects of cyanide, between those of figures 1 and 2 in which the augmentation of the expiratory contraction is either very small

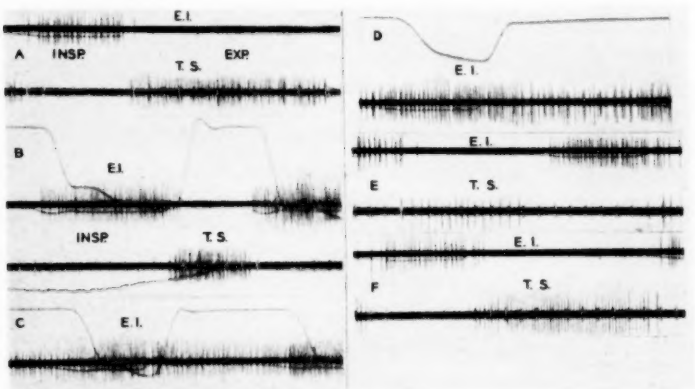

Fig. 3. Hyperpnea in which the activity of the expiratory muscles is relatively unimportant. *E.I.*, electrogram of one of the Mm. intercostales externi. *T.S.*, electrogram of M. triangularis sterni. Where the tidal air record is missing the potentials of the Mm. intercostales externi mark the phases of inspiration.

or very transient or both, are the most common (see fig. 3). The expiratory muscles stop contracting early in the period of hyperpnea—between records B and C.

For purposes of convenience we may then picture three general types of response which pass with variations from one into another: type 1—in which all of the extra energy of hyperpnea is provided by the inspiratory muscles; type 2—in which the increased activity of the expiratory muscles is important; and type 3—(intermediate) in which the augmented expiratory contractions are relatively unimportant.

Methods of breathing were more or less characteristic for the individual, yet variations in the use of muscles, presumably attributable to changing conditions, were not uncommon.

Shaping of the current respiratory act through excitation of the peripheral chemoceptors. Shaping of spinal reflexes by the continuous arrival of ever changing proprioceptive signals has been amply elucidated by Sherrington and his group (Creed, Denny-Brown, Eccles, Liddell and Sherrington, 1932). In a like way, changing proprioceptive signals from the vagal endings have been shown to exert important effects upon the current

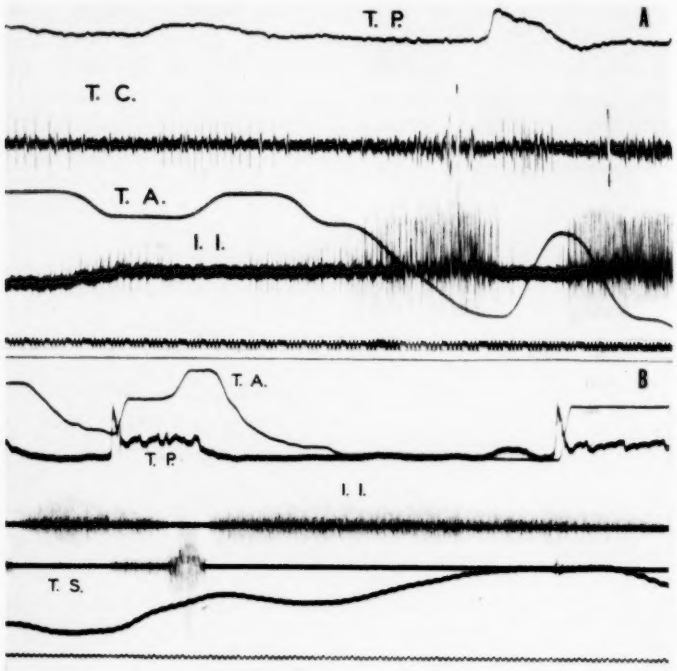

Fig. 4A. A current inspiration augmented by a timely injection of sodium cyanide. *T.C.*, electrogram of the M. transversus costarum. *I.I.*, electrogram of one of the Mm. intercostales interni.

Fig. 4B. A current expiration augmented by a timely injection of sodium cyanide. *I.I.*, electrogram of one of the Mm. intercostales interni. *T.S.*, electrogram of M. triangularis sterni.

respiratory act. Inflation of the lungs not only cuts each inspiration short (Hering and Breuer, 1868; Gad, 1880; and Head, 1889), but also accelerates the act as well (Gesell, Steffensen, and Brookhart, 1937). So far as we know, attention has never been called to effects of changing chemoceptor signals on the current respiratory act such as occur in figure 4 A. The current inspiration is suddenly augmented by a timely injection of

sodium cyanide. Reasons for believing the place of stimulation is at the outlying chemoceptor organs are that hyperpnea elicited by localized action of cyanide at the center is of smaller magnitude and of slower development than the sudden reflex deepening of respiration initiated by action at the carotid and aortic bodies (Winder, Winder and Gesell, 1933).

It seems rather significant that a similar deepening of expiration can be produced by changing the timing of injections (see fig. 4 B). In this particular observation a comparatively large injection was administered to a vagotomized animal. The result was a sharp contraction of the chest to a subnormal expiratory volume and a powerful group of expiratory potentials of the M. triangularis sterni. Obviously the firing of the respiratory mechanism is not a simple rhythmic process such as described for the heart, but an extended firing which is continually in touch with changing conditions and which shapes itself to current messages which are momentarily arriving.

Central after-discharge. Reëxamination of figure 1 reveals effects of cyanide of a more special interest. It will be seen that the inspiratory potentials of the first two breaths during hyperpnea end abruptly with the onset of expiration, but as hyperpnea progresses an after discharge follows shortly on the end of inspiration proper. At first it consists of a very short discharge of only three potentials, but eventually grows into a contraction approximating in magnitude the preceding inspiratory contraction. Not infrequently this after discharge is separated from the preceding inspiratory discharge by a silent period which varies in duration from 0.1 to a half second. The break is most obvious in the beginning of hyperpnea when the after discharge is still relatively weak and possibly easily inhibited, but as hyperpnea continues the main discharge may merge into the after discharge. Such after discharge we believe is a most important phenomenon in relation to the function of synapses and the integration of the respiratory act. It has been observed in the contraction of several inspiratory muscles—the intercartilaginous portion of the Mm. intercostales interni, the inspiratory portion of the Mm. intercostales externi, and the M. sterno thyreoideus. It seems to resemble in some respects the after discharge of outlying ganglia simultaneously reported by Bronk and Larrabee (1937).

A slight variation of results is seen in figure 3 where the after-discharge merges with the subsequent inspiratory discharge as well as with the preceding discharge, thus making the inspiratory contraction continuous. Under these conditions there is often a reciprocal relation between the activity of the inspiratory and expiratory muscles and when the continuous contractions are strong, the expiratory muscles may cease to fire entirely, as in records 3 C and D, thus indicating that after-discharge may cause inhibition of antagonistic muscles as well as suffer inhibition

from their activity. (To conserve illustration space the records of the quiescent M. transversus sterni are omitted.) As the effects of cyanide wear off, the inspiratory contractions weaken and the expiratory contractions return, beginning first with very weak contractions, as indicated by the slow firing rhythm of figure 3 E, and increasing in strength, as witnessed by the higher firing rhythm of figure 3 F.

Discussion. Judged from the point of muscular activity, the respiratory act must be considered predominantly an inspiratory mechanism. The inspiratory muscles not only provide the force for the immediate influx of air, but coincidentally, by the storage of potential energy in the lungs and torso, assure the bulk of mechanical energy for the expulsion of air during the subsequent phase of expiration. A self adjusting mechanism, therefore, comes into play with changing tidal air which automatically attains an economic use of energy, since the greater the intake during inspiration, the greater becomes the distortion and the storage of energy to reëxpel the air during expiration (Gesell, 1936). The mechanism stands in contrast to the prevailing views that greatly increased respiratory requirements are invariably met by augmented activity of the expiratory muscles which are standing in reserve.

It has been suggested (Gesell, 1937) that the contraction of abdominal muscles during the phase of expiration may not be a true respiratory act at all for these contractions are even in intensity and when prolonged by inflation of the lungs present the ear marks of tonic activity. Very significant is the fact that the "expiratory" muscles frequently do not begin to contract until the lungs have emptied and reached the expiratory volume and that frequently they exhibit no further activation during hyperpnea. Conceivably such contractions are primarily viscero-tonic reflexes of proprioceptive origin, giving the appearance of respiratory acts only because they are inhibited with each inspiration by irradiations from the central respiratory mechanism.

One of the outstanding questions in the nervous control of breathing is the integrating mechanism underlying the inspiratory barrage which fires the inspiratory muscles. How is this firing started? How is it continued? How is it brought to a close? These fundamental questions which still go unanswered lend interest to the after-discharge noted in our experiments.

Theoretically the discharge of the respiratory mechanism may arise in two ways. It may come from a reflexly automatic center, the provocative stimulus originating in the periphery or it may spring from an inherently automatic center, the provocative stimulus developing from the metabolic activity of its cells. The first view has the advantage of the ease with which a reflex respiration may be demonstrated. The second view has the prestige of long acceptance and no crucial contra-indicative evidence that inherent automaticity does not prevail.

The acceptance of inherent rhythmicity demands a mechanism of firing independent of incoming afferent signals. The rhythmic response of oxidation reduction systems to polarizing currents offers a possible cause of rhythm. A dual source of a continuous polarizing current dependent on pH and metabolic gradients has been proposed by Hertzman and Gesell (1926). The registration of slowly oscillating potentials in the brain stem of the fish comparable in rate to that of breathing (Adrian, 1931) and the demonstration of repetitive firing of nerve fibers from the flow of the current of injury (Adrian, 1930), indicate the possibility of such a mechanism. The interesting observation of Gasser (Erlanger and Gasser, 1936) that a single threshold shock applied to a nerve may give rise to a long self limited discharge during a continuous weak faradic stimulation may be a most important finding. It readily conforms with the speculation that the firing of one or more cells in the reticular formation, let us say initiated by a polarizing current, could initiate self limited discharges with the aid of the continuous impulses arising from the carotid and aortic bodies.[3]

The after-discharge which we have noted may then be due to an accumulation of central neuro humors resulting from a prolonged and powerful excitation of chemoceptor endings, the added impairment of oxidation playing a part in this accumulation; or it may be due to a modification of repetitive firing by asphyxial conditions.

SUMMARY

A study of action potentials of respiratory muscles shows that the use of these muscles is as varied in hyperpnea as in eupnea. In some individuals the extra mechanical energy required for hyperpnea is contributed entirely by the inspiratory muscles, but in most individuals hyperpnea is associated with varying degrees of increased activity of the expiratory muscles as well. The increase in activity of the expiratory muscles is usually of smaller magnitude and of shorter duration than that of the inspiratory muscles and in the later stages of hyperpnea expiration which was initially active often becomes purely passive. Active expirations

[3] This concept implies inherent rhythmicity of the central respiratory mechanism under control of afferent nerve impulses. Though such an outlook seems most useful, for the present it lacks positive proof that inherent rhythmicity exists and that rhythmicity of reflex origin does not exist. For example, can we be certain that all severed afferent channels of "deafferented" brains showing respiratory rhythm are quiescent? And perhaps more important, can we ignore the possibilities of self incorporated reflex mechanisms resident within the brain itself? Is not the presence of central chemoceptors even a plausible probability? Such structures would most certainly be active during diminished breathing following so-called peripheral denervation. A determination of the existence or non existence of sensory end organs within the brain itself comparable to those in the carotid and aortic bodies is a most essential step in the solution of problems of respiratory control.

occurring during eupnea are not infrequently abolished at the onset of hyperpnea. The mechanical advantages of such adjustments are discussed.

Selective augmentation of a current inspiration or of a current expiration may be accomplished by proper timing of intravenous injection of NaCN.

The absence of parallel changes of inspiratory and expiratory contractions in such and other observations indicates a lack of quantitative coupling of these acts.

The abrupt cessation of firing of the inspiratory muscles at the end of each inspiration, a phenomenon common for eupnea, frequently gives way during active hyperpnea to a prolonged after discharge extending into the phase of expiration.

Such after discharge may be due to several causes:

1. A central accumulation of C.E.S. (neuro humor) produced by powerful and prolonged firing of the afferent chemoceptive arcs, plus impaired oxidation.

2. An increased central excitability to reflex excitation initiated at the peripheral chemoceptors.

3. A disorganization of the normal inspiratory inhibiting mechanisms which bring the inspiratory firing to a close.

It is concluded that the firing of the respiratory mechanism is not a simple predetermined act which is finished, so to speak, at the moment of its initiation, but is modified as it unfolds: 1, by changing proprioceptive signals; 2, by changing chemoceptive signals, and 3, by a changing chemical state of the neural architectural structure itself.

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AN ADAPTATION-LIKE PHENOMENON OF ELECTRICALLY PRODUCED PHOSPHENES

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A sensation of light, known as an electrical phosphene, results when the eye or its vicinity is stimulated by an electrical current. Such phosphenes may be produced by stimulation with continuous galvanic, faradic and sinusoidal currents and by electro-magnetic fields.

Although some observers as Bouman (1), Funke (2), have described the phosphene produced by a continuous current as consisting of a flash at the make and break and a continuous light during the passage of the current, most investigators have noted a flash only on the make and break of the current. In this respect the response is similar to that in other tissues, muscle, nerve and for that matter is seen in the production of electrical energy in apparatus as in the case of mutual inductance.

The point of action of the electrical current is unknown. Many investigators believe that the point of action is in the optic nerve or retinal connecting neurones and not the retinal epithelium. This conclusion was suggested by the observation that the phosphene was not modified by light adaptation (Nagel, 3; Fischer and Hofe, 4; Achelis and Merkulow, 5) and that it seemed that the critical frequency of flickering light was lower than that of electrical phosphenes (Exner, 6; Finkelstein, 7; Filehne, 8; Bouman, 1). The latter observation was unconfirmed by Cords (9). Velhagen (10) reported the observation of electrical phosphene by a patient whose eye had recently been enucleated.

The critical frequency of electrical phosphene flicker has been reported in a range from 60 to 170 per second. As the critical frequency of light flicker is related to the brilliance of the light so is the critical frequency of phosphene flicker related to the strength of the current. Since accurate measurements were not made this point varied with different types of apparatus.

Most observers have described the critical frequency of electrical phosphenes as a fusion. This is the condition brought about by flickering light in which case when at a certain frequency the flicker disappears, a continuous light is seen. Others have only stated that the flicker disappears.

In our investigation we found that when the flicker of electrical phosphenes disappears all light disappears. In this respect the phenomenon differs entirely from the fusion produced by flickering light in part resulting from the lag in reversible photochemical changes.

The apparatus used in our study consisted of a beat frequency oscillator, without appreciable drift, calibrated for frequencies between 15 and 200 per second. The oscillations were true sinusoidal waves throughout, and the voltage output was constant in these ranges. A vacuum tube voltmeter was used to measure the r.m.s. of the output. In studying flicker threshold and strength duration curves, the output of the oscillator was amplified and after half wave rectification, the amount of current passing through the subject measured by a microammeter. Silver electrodes were used, one a larger indifferent one placed over the upper dorsal region and the other smaller one over the closed lids, or at the edge of the orbit.

Using a stimulation of a continuous current, a flash appeared only on the make or break of the current. No sensation was produced by its continuous passage. A sensation of flickering light was produced by repetitive sinusoidal or rectangular stimuli at lower frequencies. As the frequency was increased a point was reached when all light disappeared. The smaller the amount of current the lower would be the frequency at which flicker and light disappeared. But a point would be reached where no matter how strong the current was made one could see only a flash on the make or break and no flicker or light during the continued passage of the current. Up to a certain point usually about 25 cycles or 50 stimuli per second with a certain amount of current (0.1 m.a.) the flicker continued for a considerable time, numbers of seconds (20 to 40); beyond this up to the point where flicker disappeared, at about 55 to 70 cycles or 110 to 140 stimuli per second, flicker appeared on the make, lasted a second or less and disappeared. Electric current threshold for liminal flicker and frequency-duration curves were in general similar to curves obtained in studying instances of adaptation elsewhere as vibration sense, etc. The electric current threshold curve showed that with increasing frequencies increase of current was necessary to produce liminal flicker. In studying strength-duration ratios, we determined how long it took the subject to become unaware of flicker when the current was twice that necessary for liminal flicker at a given frequency. There was a consistent decrease in the length of time of persistence of flicker in going from lower to higher frequencies. More constant measurements were obtained by passing a constant amount of current, say 0.1 m.a. through the subject and noting the duration of flicker at various frequencies. Because of the very rapid disappearance of flicker at higher frequencies and the difficulty in differentiating a flicker of short duration from a flash, it was not expected to obtain curves showing strict adherence to a law. However, the curves obtained were in general similar.

Strength-liminal frequency curves resembled a hyperbola with a sudden upturn in the curve at about 80 stimuli per second when a relatively much larger amount of current was necessary to produce liminal flicker. Using 0.1 m.a. of current, strength-duration curves showed a sharp diminution in duration at about 50 stimuli a second, above which flicker persisted for but a fraction of a second (figs. 1 and 2). At first it was thought that the rapid reversal of polarity of an alternating current was responsible for the phenomenon. When, however, the current was rectified, producing unidirectional waves of a rectangular form similar results were obtained. Probably because the "on" interval was shorter in this case, the critical frequency was slightly higher.

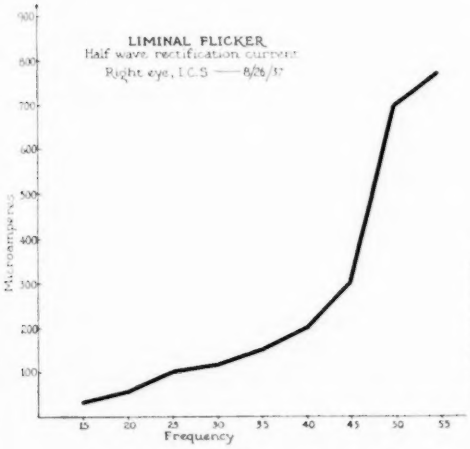

Fig. 1

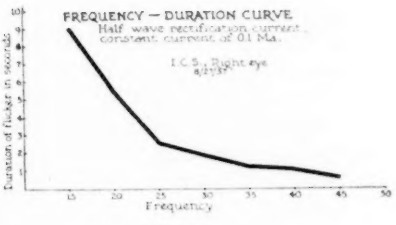

Fig. 2

We are unable to exclude effects inherent to the current. The so-called "skin" effect probably plays no part at such low frequencies. Whether a change of impedance with increasing frequencies prevents passing certain biological tissues is not known.

That the disappearance of flicker and light at higher frequencies is not the result of a stimulus of too short duration is seen from the fact that the chronaxia of the periphery of the eye is given as 1.2 to 1.8 sigma, whereas the duration of the stimulus at the time of disappearance of flicker is about 7 to 10.0 sigma.

In some ways the phenomenon resembles the apparent inhibition of Wedensky. It also resembles the "toppling down" of heat production in a nerve when stimulated with high frequencies, in which case there is no neuro-muscular junction to act as a point of impaired conduction Hill (11).

But since the rate of recovery of the phrenic nerve is such that 85 to 90 per cent recovery occurs at the end of 1 sigma and the stimuli in our case were spaced about 7 to 10 sigma apart, it does not seem that they could fall in a relatively refractory part of the nerve. The phenomenon differs from the Wedensky effect further in that when at a certain frequency just below that necessary to produce a disappearance of flicker, an increase in the strength of the current intensifies and prolongs the flicker. Furthermore, when at a higher frequency, say 160 stimuli per second, no flicker or light is observed during the continuation of stimulus, a condensor discharge produces a flash of light and when from another oscillator a current of 30 cycles per second is passed through the head, flicker phosphenes are observed.

That the phenomenon is not the result of fatigue, may be seen from the fact that the effect of disappearance of flicker and light appears within a fraction of a second after stimulation at higher frequencies. Likewise during the continued passage of imperceptible currents of high frequencies lower frequencies immediately give rise to flicker.

Action currents of the optic nerve do not differ appreciably in time relation or grouping from those in other sensory nerves (Adrian and Matthews, 12). Unfortunately it has not been possible to obtain action potentials of the optic nerve corresponding to repetitive stimuli of light beyond a relatively low frequency so that much remains to be learned concerning the mechanism of fusion.

The optic nerve as other sensory nerves possesses the function of sensory adaptation and from their studies, Hartline and Graham (13) have concluded that it may be classed with the tension and pressure receptors as opposed to tactile.

The phenomenon we are describing is quite similar to instances of sensory adaptation in other sensory organs as in the case of vibration sense. Although up to a certain frequency flicker persists, it is impossible to determine whether the number of flashes correspond to the number of stimuli or whether there is any dropping out of responses.

As in other instances of sensory adaptation, there is a relationship of electric current threshold and flicker and strength and duration of flicker. Another similarity is found in that when at a certain frequency flicker phosphenes have disappeared, closure of the lids or movement of the eyeball is followed by a burst of flickering. Cattell and Hoagland (14) found that although a constant air blast was not an effective stimulus to the skin it had a marked effect upon the response to a subsequent period of repeated stimulation. Continued stimulation of the eye by a direct current has no effect upon subsequent repetitive stimuli with sinusoidal currents, flicker phosphenes being observed as if the eye had not been previously stimulated. However, it is quite likely that the direct current produces a change only on the make or break and the stimuli are incomparable.

It is of interest to note that although vibrations of a rate of 1500 cycles per second can be felt when applied to the skin, in the case of electrical phosphenes the adaptation-like phenomenon occurs at so low a frequency. It would appear that were the retinal elements the point of stimulation, asynchronous stimulation would lead to a much higher point of critical flicker. However, without further evidence the point of action of the electrical stimulation can not be determined.

SUMMARY

Repetitive stimuli with galvanic, faradic and sinusoidal currents give rise to a sensation of flickering phosphenes until the frequency is increased to a point usually not beyond 140 per second, when sensation of light disappears. In a general way the strength of the stimulus is related to the point of disappearance of flicker, as is the strength to the duration of flicker. The phenomenon does not seem to be due to the character of the current, to fatigue or to apparent inhibition of Wedensky. It is suggested that it is the result of sensory adaptation in the optic nerve or sensory organs.

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THE RELATION OF SYMPATHETIC NERVES TO SPINAL SHOCK OF THE URINARY BLADDER OF THE CAT

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Paralysis of the urinary bladder occurs immediately after acute injuries to the spinal cord. The vesical response to stretch stimuli is lost and the bladder behaves as an inert elastic bag when fluid is introduced (Holmes, 1933). Spontaneous rhythmic contractions of the bladder are absent. The inactivity of the detrusor muscle is analogous to that of striated muscles in spinal shock. During this period chemical stimulation by a choline derivative, acetyl-beta-methylcholine chloride (Mecholyl) is ineffective in producing vesical contraction (Levin, 1938). This suggests that the detrusor paralysis is due to loss of function of the peripheral effector mechanism, as well as of central structures. There is also an increased resistance to the manual expression of urine and to the passage of a catheter.

The vesical paralysis occurs with lesions of various regions of the spinal cord, but is more marked and of longer duration when the level of lesion is carried rostrally (Levin, 1938). In cats with transection of the lower lumbar cord, the vesical paralysis usually remains complete for about a day, occasionally less than one day or as long as three days; with lesions of the thoracic cord, the paralysis remains complete from three to five days.

The present report describes a study of the relation of the lumbar portion of the spinal cord and the sympathetic nerves to spinal shock of the urinary bladder.

METHODS. Twelve adult female cats were used in this work. Studies of the urinary bladder were made either by cystometry with a water manometer and tambour recording device, or by palpation through the abdominal wall of the size of the bladder and estimation of the resistance to the expression of fluid. Cystometric study of the effects of Mecholyl were made under nembutal anesthesia, except when transection of the thoracic spinal cord rendered the animal paraplegic.

The experiments utilized four operative procedures. Two were transections of the spinal cord. This procedure, when performed at the lowest lumbar level, isolates the sacral region of the cord, the origin of the parasympathetic nerves to the bladder. The absence of paraplegia after

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this operation, except for mild weakness of the feet, indicates that the bulk of the lumbar spinal cord, which gives rise to the sympathetic vesical nerves, has been spared. Transection through the middle thoracic region interrupts cerebral pathways to both the sympathetic and the parasympathetic vesical mechanisms in the spinal cord.

In the other two operations, the sympathetic nerves to the bladder were bilaterally sectioned. This was usually performed through a unilateral retroperitoneal approach to the sympathetic ganglia and hypogastric nerves which lie in front of the lower lumbar vertebrae. These nerves contain the postganglionic sympathetic fibers to the bladder. In other cases, the preganglionic fibers were cut within the spinal canal, after an extensive lumbar and lower thoracic laminectomy, by section of all the lumbar and lower thoracic ventral roots.

Observations. The first experiment to be described deals with the effects of consecutive transections of the spinal cord, first in the lower lumbar region, and then in the thoracic region. The first operation, isolating the conus terminalis and its nerve roots, produced the state of spinal shock in the bladder. The detrusor was paralyzed and failed to react to Mecholyl (fig. 1 A); the bladder resisted expression. As the sacral reflexes returned and the detrusor became active, Mecholyl produced vesical contraction with micturition (fig. 1 B and C). After 25 days, an interval sufficient to permit full recovery of reflex activity of the isolated portion of the spinal cord, the thoracic cord was then transected. The effect of this operation in the cats which had recovered from the spinal shock was the same as when the thoracic transection is performed in normal animals. The bladder again became inert and failed to react to Mecholyl (fig. 1 D). After a few days detrusor activity returned and a slow rise in pressure resulted from the administration of Mecholyl (fig. 1 E), but it was inadequate to initiate micturition. Expression of the bladder, which had become easy with recovery from the first spinal shock, again became difficult, a pressure of 43 cm. of water now being required for the release of fluid around the catheter. On the fifth day after the thoracic transection periodic micturition was established, the bladder became smaller, waves of detrusor contractions were present and fluid escaped at a low pressure (12.5 cm.). Mecholyl now produced micturition with ease (fig. 1 F).

This experiment was performed on six cats with consistent results. In some cases the observations were made by simple observation of vesical activity in unanesthetized cats. The intervals between the two transections varied from 7 to 43 days.

The influence of previous sympathectomy upon the effects of the second (thoracic) transection was then observed. In a typical case, after recovery from the spinal shock resulting from isolation of the sacral cord (12 days after the operation) both lumbar sympathetic chains were resected. Five

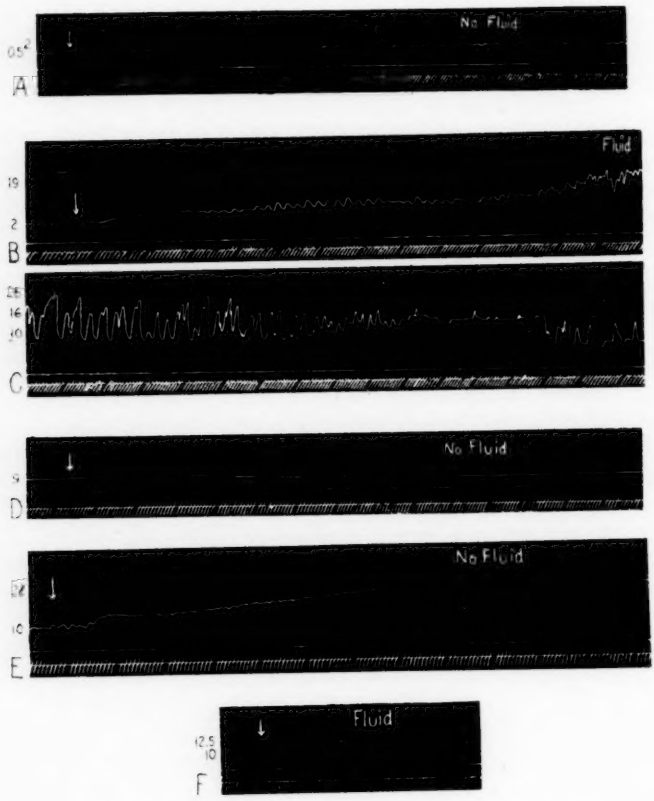

Fig. 1. Responses of the urinary bladder of a cat to Mecholyl (in doses of 5 mgm.) at intervals after transections of the lumbar and thoracic spinal cord. Nembutal anesthesia. The time of injection of the drug is indicated by the arrow. A, 1 day after transection of the lower lumbar cord, Mecholyl produced only a slight rise in intravesical pressure, from 0.5 to 2 cm. of water, no fluid escaping. Slight rhythmic variations in pressure appeared. B and C are consecutive portions of a record made 3 days after operation, showing a slow rise in pressure and marked variations. Fluid began to escape at a pressure of 19 cm. of water, continued when the pressure dropped to 16 cm. and still later at a pressure of 10 cm. The plateau in the latter part of C is an artefact. D, 3 hours after transection of the thoracic cord (25 days after the lower transection), Mecholyl produced no rise in pressure. E, 3 days after the thoracic transection, there was a slow rise in pressure to 22 cm., inadequate to effect micturition. F, 5 days after the thoracic transection, when automatic micturition was established, a rise of pressure to 12.5 cm. was sufficient for micturition. Time in 5 seconds.

days later the bladder was small and reacted satisfactorily to Mecholyl, fluid escaping at a pressure of 15 cm. of water. After two more days the spinal cord was transected in the upper thoracic region. The absence of spinal shock was first indicated by the fact that the cat defecated frequently during the following night. Graphic records of vesical activity were obtained on the day after operation. With the bladder partially filled, there were spontaneous rhythmic contractions of large amplitude (fig. 2). The rapidity of the changes in intravesical tension indicated that the detrusor contraction was well coördinated. The waves occurred at intervals of 65 seconds. Five milligrams of Mecholyl were injected subcutaneously and produced a response which was evident within one and a half minutes. There was a rise in the resting pressure between the waves from 11 cm. to 19 cm. of water. The frequency of the vesical waves also

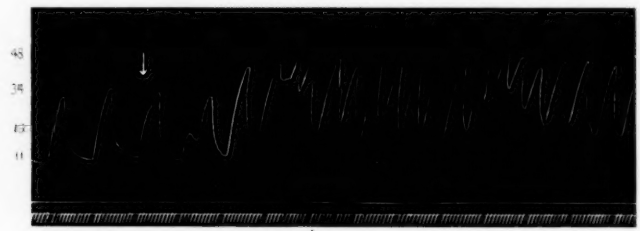

Fig. 2. The effect of Mecholyl 1 day after thoracic cord transection in a cat with isolated sacral cord and sympathectomy. There was no spinal shock in the bladder: spontaneous waves were present, the pressures varying from 11 to 34 cm. Mecholyl (5 mgm.) produced a rise in resting pressure to 19 cm. and the waves became more frequent, the highest pressure being 48 cm. Micturition occurred. Time in 5 seconds.

increased so that the interval between them was now 30 seconds. The variation in pressure in the single waves was also increased. Micturition now occurred, resulting in evacuation of the bladder. The pressure at which fluid escaped was rather high (37 cm.), corresponding with a moderate resistance to the expression of urine. In other cases the pressure at which fluid escaped, after the series of operations, remained low, as low as 16 cm.

Another experiment consisted of section of all the lumbar and lower thoracic roots following recovery of vesical reflexes after isolation of the sacral cord. Further injury to the conus was avoided during the operation. On the day after the second operation the bladder was still moderately large but expression was easy; fluid escaped around the catheter when the pressure was only 10 cm. Mecholyl produced a vesical contraction that was adequate for micturition. The prevention of spinal shock

could thus be effected by interruption of the preganglionic sympathetic fibers as well as by section of the postganglionic nerves.

The next observations dealt with the influence of sympathectomy upon the spinal shock resulting from transection of the thoracic cord in normal animals. The cord lesion was made three days after resection of both lumbar sympathetic chains, the bladder now being small and within the pelvis. On the following day the bladder was enlarged and resisted expression. A pressure of 40 cm. was required for the escape of fluid around the catheter. Mecholyl was ineffective in producing vesical contraction, although satisfactory absorption was indicated by a good general effect of the drug. It was evident that sympathetic denervation does not prevent shock of the bladder resulting from lesions of all portions of the spinal cord, but only that dependent upon abnormal function of the lumbar cord.

Discussion. The results of these experiments show that spinal shock of the urinary bladder may be produced twice in the same animal, first by isolation of the sacral cord (origin of the parasympathetic vesical nerves) and second by isolation of the lumbar cord (origin of the sympathetic nerves). In either case the detrusor muscle becomes inactive to reflex stimulation by stretch and to chemical stimulation by choline derivatives; there is also resistance to the manual expression of urine. If the sympathetic vesical nerves are resected before the second transection, which isolates the lumbar spinal cord, the second period of shock does not develop. Sympathectomy itself does not produce paralysis of the bladder similar to that which occurs in shock, but rather enhances vesical emptying (Langworthy, Reeves and Tauber, 1934). The vesical changes in shock are similar to those found by Elliott (1907) after prolonged electrical stimulation of the hypogastric nerves; he described "such flaccidity that the bladder never regains its tone, not even after effective stimulation of the pelvic nerves." One wonders, then, whether the sympathetic vesical reflexes are not still active in the period of shock following transection of the spinal cord.

SUMMARY

Spinal shock appears in the urinary bladder after transection of the spinal cord at the lowest lumbar level, isolating the sacral region. After the recovery of reflex activity of this region, producing automatic micturition, a second transection of the thoracic spinal cord causes the state of shock to reappear. This effect of isolation of the lumbar spinal cord does not occur if the animal has been sympathectomized.

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THE PANCREAS AND THE DEPOSITION OF FAT IN THE LIVER

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The report by Dragstedt and his colleagues (1936) that an alcoholic extract of pancreas added to the diet of depancreatized dogs receiving insulin permitted survival of the animals for prolonged periods and prevented the infiltration of their livers with fat was of great interest to us. It had previously been shown in the Department of Physiology and in these laboratories that choline exerts similar effects in depancreatized dogs (Best, Ferguson and Hershey, 1933). It has been known also from the work of Best and Huntsman (1935) and subsequently that of Channon and Wilkinson (1935) that casein and other dietary proteins exerted an effect similar to that of choline in normal rats. Furthermore, it has been shown that betaine (Best and Huntsman, 1932) possessed a choline-like action on liver fat. Dragstedt and his co-workers believe that the potency of their extract is not attributable to the small amount of choline which it contains. It would provide only an insignificant amount of protein in the doses which they used and there is little likelihood that it contains large quantities of betaine. Quite recently MacKay (1937) has reported that extracts made by Dragstedt's procedure prevent the deposition of fat in the livers of rats when these animals are maintained on a diet deficient in choline and other lipotropic factors.

Soon after Dragstedt's preliminary report appeared we conducted experiments on rats in which small amounts of fresh beef pancreas, liver and muscle were added to diets low in choline. The results did not indicate that pancreas contained any specific substance which, under these experimental conditions, affected liver fat. More recently using a pancreatic extract ("lipocaic") prepared by Eli Lilly and Company and tested by Doctor Dragstedt, we have conducted further experiments using large groups of rats. The results of these latter studies provide no evidence that the extract contains any substance affecting the deposition of liver fat in addition to choline and the unknown factor or factors associated with protein.

METHODS. The procedure used for estimation of fat in the liver and the details of the care of the animals are essentially similar to those we have described elsewhere.

EXPERIMENTAL RESULTS AND COMMENTS. In the first experiment five groups of rats with 20 animals in a group were used. The first group was placed on the basal diet which had the following composition. A meat powder made by extracting beef muscle with strong alcohol (MacLean, Ridout and Best, 1937) provided 10 per cent by weight of the diet. Beef fat 40 per cent, sucrose 43 per cent, salt mixture 5 per cent and agar 2 per cent were used. Four units of vitamin B_1, 75 units of vitamin A and 8 units of vitamin D were added to every 10 grams of food. The duration of the experiment was 21 days. The second group received the basal diet plus 1 gram of fresh beef pancreas daily; the third group, the basal diet plus 1 gram of fresh beef liver; the fourth group, the basal diet plus 1 gram of fresh beef muscle and the fifth group, the basal diet plus a small amount of choline. The results of these experiments are summarized in table 1. They show that the liver exerted quite as definite an effect as

TABLE 1

EXPERIMENT	CHANGE IN WEIGHT	AVERAGE DAILY FOOD INTAKE	LIVER FATTY ACIDS	
			Total	Per cent
	grams	*grams*	*grams*	
Basal diet	+2	9.0	3.01	22.4
Basal diet + 1 gram fresh beef pancreas daily	+16	9.2	0.93	11.8
Basal diet + 1 gram fresh beef liver daily	+40	9.7	0.71	8.9
Basal diet + 1 gram fresh beef muscle daily	+10	9.4	1.46	17.6
Basal diet + 5.6 mgm. choline daily	+1	9.4	0.66	9.7

the pancreas while the effect of muscle was appreciably less than that of the other tissues. The choline equivalent of liver is definitely greater than that of pancreas while that of muscle is the least of the three. The choline equivalent is calculated in the following manner. The protein nitrogen in the material was estimated and the protein calculated from this value. The choline equivalent of each gram of protein was taken as 5 mgm. This is the approximate choline equivalent of 1 gram of casein. The choline content of the extract was determined as previously described by Fletcher, Best and Solandt (1935). The sum of the choline content and the choline equivalent of the protein gives the choline equivalent of the material. The accuracy of this calculation is admittedly not great but, in the absence of better figures, it seemed reasonable to base the choline equivalent of the protein in fresh pancreas, liver, muscle or in the pancreatic extract on that of casein. It is quite possible that the protein of pancreas has a greater lipotropic effect than the alcohol and ether extracted casein. Protein of liver, pancreas and muscle prepared in the same way as the "fat and vitamin free" dietary casein has not as yet been

tested for lipotropic properties. In the above experiment the choline equivalent of the amount of pancreas used was 4.46 mgm. while that of the liver was 6.02 mgm. We accordingly decided to feed 5.6 mgm. of choline to one group of animals. This amount of choline exerted approximately the same effect on liver fat as did the pancreas and liver.

In a second experiment an extract prepared by Dragstedt's procedure and found by him and his colleagues to be active in depancreatized dogs was used. The duration of the experiment was 21 days. Three groups of 15 animals each were used. The first group of animals received a basal diet of the same composition as that previously described. The second group received 369 mgm. of pancreatic extract in 10 grams of food. While this extract contains only small quantities of choline, there is a relatively large amount of protein (17 per cent). It is difficult accurately to estimate the choline equivalent but we decided to feed 1.5 mgm. of choline per rat per day to the third group of animals. The results which are summarized

TABLE 2

EXPERIMENT	CHANGE IN WEIGHT	AVERAGE DAILY FOOD INTAKE	LIVER FATTY ACIDS	
			Total	Per cent
	grams	*grams*	*grams*	
Basal diet	+5	10.0	1.52	17.0
Basal diet + 369 mgm. pancreatic extract ("lipocaic")	+10	10.0	0.70	9.6
Basal diet + 1.5 mgm. choline daily	+17	10.0	0.86	10.6

in table 2 show there is no significant difference between the effect of the pancreatic extract and the small amount of choline which was administered to the third group of animals.

In the third experiment further quantities of the same pancreatic extract were administered. The basal diet was the same as before. The duration of this experiment was 20 days. The rats were divided into 5 groups of 15 animals each. The first group was placed on the basal diet, the second group received 1.96 mgm. of choline daily in addition to the basal ration. The third group received the basal ration and 5.0 mgm. of choline daily. The fourth group received the basal diet and 875 mgm. of the pancreatic extract daily. The fifth group received the basal ration and 1.05 gram of casein daily. The choline equivalents, therefore, of the supplements added to the diet for the various groups were as follows: group I, 0.0 mgm.; group II, 1.96 mgm.; group III, 5.00 mgm.; group IV, 2.06 mgm.; group V, 5.20 mgm. In these experiments, as in the ones we have previously reported, there is considerable individual variation in the figures. For this reason it is unsafe to draw conclusions from the results

obtained on three or four animals. The results given in table 3 certainly do not indicate that pancreatic extract contains any factor affecting liver fat in addition to choline and the choline-like substances associated with protein. It would appear that this particular sample of casein is quite as effective, gram for gram, as the dried pancreatic extract.

Discussion. The nature of the substances which are responsible for the effect which the feeding of pancreas produces on the deposition of fat in the livers of depancreatized dogs is not as yet completely known. Since choline has been shown to be an effective lipotropic substance and since all tissues contain appreciable amounts of it, a part of the effect of pancreas may be ascribed to its choline content. Various betaines may also be present in this tissue. Furthermore, the protein in pancreas may have associated with it a substance or substances similar to that which is present in dietary casein and some other proteins. To these three possibilities

TABLE 3

EXPERIMENT	CHANGE IN WEIGHT	AVERAGE DAILY FOOD INTAKE	LIVER FATTY ACIDS	
			Total	Per cent
	grams	*grams*	*grams*	
Basal diet	−1	10.0	1.22	14.2
Basal diet + 1.96 mgm. choline daily	0	10.0	0.99	11.9
Basal diet + 5.0 mgm. choline daily	+6	10.0	0.60	7.7
Basal diet + 875 mgm. pancreatic extract ("lipocaic") daily	−8	10.0	0.75	10.1
Basal diet + 1.05 grams casein daily	+25	10.0	0.78	8.8

a fourth may be added when depancreatized dogs are used as test animals. It has been shown by various workers that the absorption of protein and fat are incomplete in the depancreatized dog. It is very difficult to make a satisfactory estimation of the amount of choline and other lipotropic factors which are lost in this way or of the increase in the amount retained when pancreas is added to the diet. Some time ago we made choline estimations on the excreta of depancreatized dogs under various conditions but these results did not furnish complete information since the amounts of the non-choline lipotropic factors excreted by the animals were unknown. It will be appreciated that, in the light of the above facts, we have never suggested that all the effects on the liver fat of depancreatized dogs produced by the addition of pancreas to the diet could be attributed to the choline contained in the tissue.

The factors which affect the deposition of fat in the liver of the depancreatized dog may not be identical with those which control this process in the normal rat. Our results in the latter species, therefore, while they

lend no support to the views of Dragstedt and his collaborators, do not prove that the pancreatic extract does not contain some new factor affecting the liver fat of depancreatized dogs. We have attempted recently, however, to advance the general problem of the factors which affect liver fat by using large numbers of small laboratory animals. There is no doubt that the use of depancreatized dogs presents many problems (see also Chaikoff and Kaplan, 1937) which can be avoided by studying small normal animals.

The results discussed above appear to prove that no new specific factor affecting the liver fat of rats is present in the pancreatic extract. It is difficult to reconcile our results with the interpretation which MacKay has placed upon those which he has obtained. This investigator, however, has not as yet studied the effect of the choline equivalent of his pancreatic extracts on large groups of animals and may not fully appreciate the profound effects one or two milligrams of choline in the daily ration may exert on the liver fat of the rat. In a personal communication MacKay reports that a sample of casein tested in his laboratory does not exert as much lipotropic effect as the pancreatic extract. Here again our results suggest a different conclusion, but the same samples of casein and extract were not used in the two investigations In any case it will be interesting to compare the choline equivalents of various samples of casein with that of the pancreatic extract. It would not appear probable to us that a dose of choline, equal to the choline equivalent of the pancreatic extract as determined in rats, would exert an appreciable effect on the liver fat of depancreatized dogs. Since Dragstedt used a daily dose of from 1.0 to 1.5 grams of pancreatic extract, this would provide only the equivalent of from 3 to 4 mgm. of choline. It would therefore appear that, if the results of Dragstedt and his collaborators are confirmed, the pancreatic extract contains a substance affecting the deposition of liver fat in the depancreatized dog but not in the normal rat. Lecithin and choline have been shown to exert similar effects in the two species.

SUMMARY AND CONCLUSIONS

1. The results of investigations in which fresh beef pancreas, liver and muscle are added to a diet poor in choline and other lipotropic factors do not suggest that pancreas contains any new specific factor affecting the deposition of liver fat in white rats.

2. A sample of a pancreatic extract ("lipocaic") reported by Dragstedt and his collaborators to be potent in depancreatized dogs exerted only the lipotropic effect in rats which was predicted from its choline and protein content.

3. It is considered unlikely that an amount of choline equivalent to the total lipotropic value, as determined in rats, of the dose of pancreatic

extract used by Dragstedt would have any effect on the liver fat of depan creatized dogs.

4. The lipotropic effect of the pancreatic extract was approximately the same as that of a sample of dietary casein.

5. The bearing of these results on the general problem of deposition of fat in the liver has been discussed.

ADDENDUM

Since this paper was sent to press there have been two further communications on the subject. The results we have obtained on feeding raw pancreas to rats are in agreement with those reported by Aylward and Holt (J. Biol. Chem. **121:** 61, 1937). Shapiro and Wertheimer (Nature **140:** 771, 1937) have arrived at different conclusions. Their note, however, does not provide sufficient detail to permit criticism of individual experiments.

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THE EFFECTS OF CASTRATION, THEELIN, TESTOSTERONE AND ANTUITRIN-S ON THE LIPOIDS OF BLOOD, LIVER AND MUSCLE OF GUINEA PIGS

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A relation between sexual activity and the metabolism of fat has been recognized for a long time. It has been known that obesity frequently increases after castration or the physiological cessation of sexual functions. The big advance in the chemistry of sexual hormones has made possible investigations with definite substances and exact doses. For this reason, it seemed worth while to examine in a series of experiments the relation of sexual hormones and lipoids of whole blood, liver and muscle. Doses used in all these experiments were not large in order to imitate as well as possible the changes of sexual activity as it occurs in the life cycle.

EXPERIMENTAL. As experimental animals, guinea pigs were selected. For at least three weeks before experimentation, and after operation or injections, they were on a standard diet of oats, lettuce and carrots fed ad libitum without any additional water. All animals were kept under uniform conditions, i.e., each was in a separate cage, all cages were of exactly the same size, the room temperature varied between 20 and 24°C. All animals were weighed once a week and were examined minutely to insure absolutely controlled conditions. Oestrous cycles of the females were observed during the whole duration of the experiments.

Twenty-four hours before killing an animal, food was removed from its cage. Each animal was killed by a blow on the head. Immediately after death, blood was drawn by puncture of the heart and then the liver and muscle samples were removed. The muscle sample was always taken from the same region of the right hind leg. All animals were autopsied and any which showed pathological changes were discarded. The completeness of operation was also controlled by the autopsy.

The whole liver was weighed within a maximum of one hour after its removal from the guinea pig. A weighed sample of about 1.0 to 2.0 grams of liver or of 3.0 to 6.0 grams of muscle was ground with fine Ottawa-sand and immediately covered with alcohol and ether mixture (1). For extraction it was refluxed for at least thirty minutes with each of three different samples of approximately 50 cc. of alcohol-ether mixture. After refluxing,

filtration and washing of the precipitate, the final volume was made up to 200 cc. The extracted tissue, sand and filter paper were used to determine the weight of tissue, unextracted by alcohol and ether, and dried at 100 degrees to constant weight (4). Duplicate aliquots of the alcohol-ether extract were used to determine fatty acids by the method of Man and Gildea (1), total cholesterol and lipoid phosphorus by the method of Man and Peters (2, 3). A modification of Ralli (4) was used in the gravimetric determination of cholesterol. After constant weight of the Jena funnel and digitonide precipitate had been determined, the cholesterol digitonide was dissolved with three portions of boiling methyl alcohol which were filtered through the funnel by suction. The funnel was then washed with boiling distilled water, dried and weighed. The difference in weight was assumed to be the weight of cholesterol digitonide. Thus any non-cholesterol contaminating substances were left in the funnel and were not included in the weight of the cholesterol digitonide.

Eisenman's methods were used to defibrinate the whole blood (5) and to determine hematocrits and dry weights (6). Fatty acids and cholesterol of whole blood were evaluated by the methods of Man, Gildea and Peters (1, 2), adding to the cholesterol technique the modification of Ralli (4) described in regard to the determination of liver and muscle cholesterol.

Nine different groups of animals were used for experimentation: normal adult animals 6 to 9 months old, immature animals, animals 13 to 25 months old, castrated males, castrated females, males injected with testosterone,[1] females injected with theelin,[2] males injected with antuitrin-S, females injected with antuitrin-S. All injections were made subcutaneously. The theelin and antuitrin-S were aqueous solutions, while the testosterone was in ethylene glycol. Doses of the hormones and the duration of all experimental procedures are given in the table.

Results. In figure 1 are shown graphically the average figures for each group of animals of blood, liver and muscle lipoids. To make the columns somewhat comparable in these different tissues and between fatty acids, cholesterol and lipoid phosphorus, the minimum and maximum values for each lipoid in each tissue of all the guinea pigs were taken as limits. Then on this scale the average value for each tissue lipoid in each of the nine experimental groups of animals has been plotted. In other words, the columns represent percentage changes between minimum and maximum values.

In table 1 are given the actual experimental figures on the three age

[1] Testosterone used in these experiments was supplied by the Schering Corporation, Bloomfield, N. J. through the kindness of Doctor Schwenk.

[2] Theelin and antuitrin-S used in these experiments were supplied by Parke, Davis and Company, Detroit, Michigan through the kindness of Doctor Kamm.

groups of untreated animals. The variations from animal to animal were marked and for this reason it seems more exact to present data for individual animals rather than average figures for each group. Examples of these wide differences occur between the blood fatty acids of animals 15 and 20, 21 and 26, between the blood cholesterols of animals 5 and 6, 25 and 29, between the liver fatty acids of animals 1 and 2, 9 and 10, 14 and

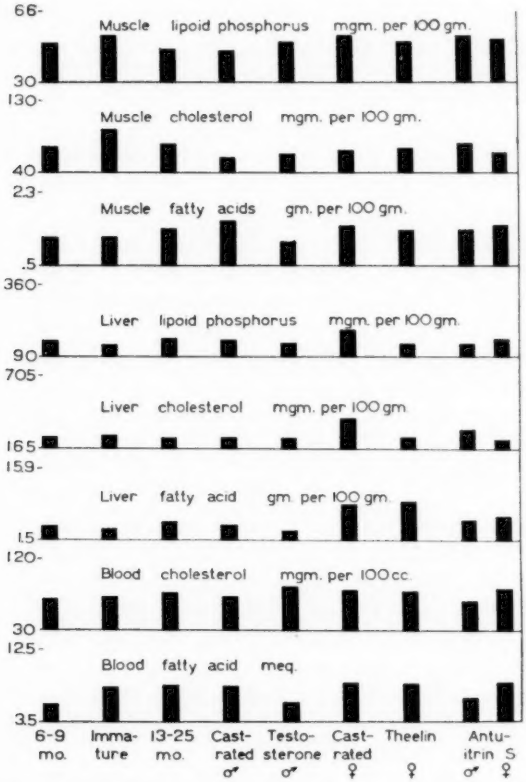

Fig. 1. Average lipoids in blood, liver and muscle of guinea pigs. (Liver and muscle fatty acids expressed in tripalmitin.)

19, 25 and 29, between the muscle cholesterol of animals 1 and 9, 22 and 25.

It is difficult to compare these values with determinations of the tissue lipoids of normal guinea pigs because such published data are rare, but the values for liver lipoids agree fairly closely with values obtained by Ralli on liver lipoids of normal dogs (8).

TABLE 1

Lipoids of blood, liver, and muscle of normal guinea pigs

NUMBER	SEX	AGE	BODY WEIGHT	BLOOD		LIVER					MUSCLE		
				Fatty acids	Total Cholesterol	Weight	Body weight / Liver weight	Fatty acids in tripalmitin	Total cholesterol	Lipoid phosphorus	Fatty acids in tripalmitin	Total cholesterol	Lipoid phosphorus
I. Animals 6–9 months old													
		months	*grams*	*m.-eq.*	*mgm. per 100 cc.*	*grams*		*grams per 100 grams*	*mgm. per 100 grams*	*mgm. per 100 grams*	*gram per 100 grams*	*mgm. per 100 grams*	*mgm. per 100 grams*
1	♀	6	546			15.1	36.1	6.6	272	135	1.9	102	58
2	♀	6	641	5.4	79	25.8	24.8	1.9	213	134	0.9	74	57
3	♀	6	625	6.7	80	22.6	27.6	2.9	249	160	1.0		57
4	♀	6	495	4.5	71	19.1	25.9	1.9	234	141	1.1	78	45
5	♀	6	488	5.6	101	12.2	40.0	5.7		168	1.2	74	53
6	♂	7	603	6.8	47	14.1	42.7	4.6	271	142	1.8	78	57
7	♂	7	634			19.4	32.6	2.7	209	130	0.6	65	42
8	♂	7	622	5.1	76	18.1	34.3	2.3	206	165	1.0	65	49
9	♂	7	685	5.6	74	16.9	40.5	2.1	223	168	0.8	42	34
10	♂	9	609	6.4	64	16.5	36.9	6.6	248	148	1.2	64	55
11	♂	9	620	5.7	55	20.4	30.4	6.1	277	131	1.0	61	48
12	♂	9	533	5.2	48	14.9	35.7	6.3	276	137	1.5	73	50
II. Immature animals													
13	♀	2	261	7.1	65	8.2	31.8	5.5		151	1.7	91	65
14	♂	2	265	9.9	99	7.5	35.3	6.7	249	139	1.1	90	55
15	♂	2	255	10.1	85	7.4	34.4	2.5	299	167	1.1	104	54
16	♀	2	279	7.8	64	16.7	16.6	1.7	198	121	1.6	94	51
17	♀	2	247	8.7	52	6.5	38.0	2.0	310	131	0.8	128	60
18	♂	2	258	6.7	68	6.3	40.9	2.0	295	128	0.6	85	40
19	♂	1	289	7.2	63	16.4	17.6	1.3	186	95	1.3	73	48
20	♀	2	337	4.6	77	10.8	31.2	8.1	277	141	1.4	76	59
III. Animals 13–25 months old													
21	♀	13	886	5.3	89	22.0	40.3	4.0	270	159	1.3	69	41
22	♀	13	846	5.8	98	24.6	34.3	2.1	266	172	0.6	48	33
23	♀	17	867	7.3	94	26.1	33.2	5.3	213	169	2.2	71	52
24	♀	25	907	8.5	77	26.1	34.7	8.1	244	143	2.3	89	47
25	♂	14	775	7.1	100	19.3	40.2	2.2	201	164	1.3	112	51
26	♂	14	651	10.2	59	15.5	42.0	3.3	255	172	0.6	67	41
27	♂	19	772	8.4	65	18.7	41.3	4.6	218	157	1.3	69	52
28	♂	19	661	10.0	78	18.9	34.9	5.6	296	153	1.2	83	57
29	♂	19	804	9.0	34	22.9	35.1	10.8	244	143	1.5	70	52

From data on normal control animals in group I it can be seen that the blood cholesterol of females ranges from 71 to 101, with an average of 83, of males from 47 to 76 with an average of 61 milligrams per cent. There was no variation in blood cholesterol or fatty acids of females in relation to the oestrous cycle, which confirms the findings of Man and Gildea in relation to the seasonal variations of serum lipoids in women (7). Normal females seemed to have proportionately heavier livers than males (consequently the factor: body weight over liver weight is smaller for females). The variations in fatty acids in the livers of normal animals were fairly large. Little variation in liver cholesterol and phosphatides occurred. Variations in fatty acids and cholesterol of muscle were apparent; but with one exception the phosphatides of muscle were fairly constant. The cholesterol in muscles of females was higher than that in males and the data suggest that this might also be true in regard to phosphatides.

The fatty acid variations in the liver of the immature animals were even greater than in the normal adult animals. Cholesterol differences in blood and liver were also large. These variations might suggest that the immaturity of these animals is associated with instability of fat metabolism and this instability may in turn be related to the elevation of the fatty acids above the level in the blood of adult animals. The liver weights of females seemed to be very slightly greater than in males in proportion to total body weight. In the muscles there was a higher percentage of cholesterol than in the normal controls, and the figures for the females appeared to be slightly higher than for the males. Phospholipoids in muscle were slightly higher than in the adults.

The fatty acids of blood of the older animals, 13 to 25 months, were markedly higher than of the animals 6 to 9 months old. The cholesterolemia of the females was somewhat greater than that of the males. Fatty acid and, to a much less degree, phosphatides in the livers seemed to be somewhat increased if compared with the data on group I. The factor body weight over liver weight was higher in males than in females.

The blood fatty acids of 4 males, 2 of which had been castrated five months, and 2 three months before determination of tissue lipoids were only slightly increased beyond those of animals of group I. Fatty acids of the livers were increased only in one animal. Cholesterol in muscles was slightly decreased.[3]

Fatty acids in the blood of four females castrated $1\frac{1}{2}$ to $3\frac{1}{2}$ months before determination of tissue lipoids were increased. Fatty acids of liver were greatly increased in one animal, increased in another and within normal limits in the other two animals. The liver cholesterol of one animal was also markedly elevated while those of two were somewhat elevated. The

[3]Data on individual castrated animals are omitted for the sake of brevity, but can easily be sent to any inquirer.

TABLE 2

Lipoids of blood, liver and muscle of treated animals

NUMBER	AGE	DAILY DOSE	NUMBER OF DAYS	BODY WEIGHT	BLOOD		LIVER					MUSCLE		
					Fatty acids	Total cholesterol	Weight	Body weight / Liver weight	Fatty acids in tripalmitin	Total cholesterol	Lipoid phosphorus	Fatty acids in tripalmitin	Total cholesterol	Lipoid phosphorus
Females injected with theelin														
	mos.			*gram*	*m.-eq.*	*mgm. per 100 cc.*	*grams*		*grams per 100 grams*	*mgm. per 100 grams*	*mgm. per 100 grams*	*grams per 100 grams*	*mgm. per 100 grams*	*mgm. per 100 grams*
30	7	2 × 10 I.U.*	35	522	5.7	80	19.6	26.6	3.2	266	125	1.1	73	49
31	7	2 × 10 I.U.	28	482	7.8		13.5	35.7	5.0	285	152	1.2	47	51
32	14	2 × 10 I.U.	38	590	5.8	106	13.4	44.0	5.7	223	153	1.8	78	57
33	14	2 × 10 I.U.	31	622	8.5	95	17.0	36.6	12.1	271	142	1.1	63	45
34	8	2 × 20 I.U.	31	525	8.0	56	15.2	34.5	14.0	216	141	1.5	71	60
35	8	2 × 20 I.U.	31	542	12.1	64	14.0	38.7	11.9	214	130	1.4	86	51
36	14	2 × 20 I.U.	31	801	9.3	75	27.8	28.8	12.2	288	145	1.4	79	46
Males injected with antuitrin-S														
37	15	10 U.†	35	792	4.9	80	18.9	41.9	6.9	399	150	1.3	56	58
38	7	10 U.	31	686	7.3	69	20.4	33.6	2.2	236	132	1.2	67	50
39	8	10 U.	38	737	4.1	35	22.2	33.2	3.9	350	142	1.1	88	44
40	15	10 U.	28	816	6.1	47	25.0	32.6	4.4	332	144	1.5	90	62
41	7	10 U.	28	607	5.7	98	15.7	38.7	2.9	371	144	1.3	83	58
42	7	10 U.	28	588	6.3	78	14.5	40.6	5.6	275	158	1.2	97	56
43	14	20 U.	40	847	7.3	77	26.7	31.7	12.9	221	128	1.6	71	49
44	14	20 U.	40	923	9.8	63	21.2	43.5	5.1	282	123	1.8	68	51
Females injected with antuitrin-S														
45	7	10 U.	29	468	8.4	82	10.1	46.3	2.8	206	184	1.0	48	45
46	7	10 U.	35	507			14.8	34.3	9.2	267	166	1.6	67	58
47	14	10 U.	29	771	9.5	88	19.2	40.2	7.7	239	145	2.3	78	64
48	15	10 U.	35	732	6.0	109	20.0	36.6	4.7	249	149	1.4	50	50
49	7	20 U.	35	540			17.3	31.2	8.3	234	144	1.4	59	51
50	7	20 U.	35	550	9.1	73	17.2	31.9	6.4	275	164	1.4	81	52
51	14	20 U.	35	726	8.7	66	20.6	35.2	5.1	167	148	1.6	70	45

* International unit.

† Unit.

liver phosphatides showed no variations except in one animal. In muscle, the cholesterol was slightly decreased if compared with the females of group I.[3]

In the blood of two of the four males injected with 20 gamma of testo-

sterone for 28 to 35 days, fatty acids and cholesterol were increased. Three control animals injected for the same time with the same amount of pure ethylene glycol showed no variation from non-injected normal males.

In table 2 are given the data on tissue lipoids, the dosage and length of treatment of females injected with theelin, and of both males and females injected with antuitrin-S. Individual data on the females injected with theelin show that the fatty acids of the blood and especially of the liver were the highest in any of the groups. The increases were more marked in the animals given 40 international units per day than in the animals injected with half this quantity of theelin.

Fatty acids in the blood and muscles of males injected with antuitrin-S were increased in some cases especially when larger doses were employed. In only one case was there an elevation in the liver fatty acids. The liver cholesterol was increased in four of six animals injected with smaller doses of antuitrin-S but not elevated in two animals injected with larger doses of this hormone. That the injection of a single hormone may apparently not alter lipoid metabolism is not surprising because physiological balance is maintained by complicated systems in which increase of one hormone may be attended by an increase in an opposing endocrine activity. The effects might even be opposite to what was expected if the opposing mechanism were too highly stimulated by large doses of an hormone. This may explain why smaller rather than the larger doses of antuitrin-S apparently altered liver cholesterol.

Females injected with antuitrin-S exhibited increases in blood fatty acids which exceeded the elevation in blood fatty acids of males injected with this same hormone. The liver fatty acids were elevated in three of seven female animals. Phosphatides in liver were very slightly increased.

Hematocrit determinations showed that the changes in blood lipoids were real and not due to alterations in the ratio of cells to plasma. In every sample of blood, liver and muscle, water content was checked by the methods previously described. The results are not included in the charts, but they showed only that the males injected with testosterone and the females with larger doses of theelin had increases in the water contents of the livers. Parhon and Cahane (9) found also an increase in liver water after injection of folliculine.

CONCLUSIONS

Fatty acids and cholesterol in the blood, liver and muscle and lipoid phosphorus in the liver and muscle of 63 guinea pigs have been determined. Non-treated animals were divided into three age groups classified as immature, 6 to 9 months and 13 to 25 months old. Treated animals were castrated or were given testosterone, theelin or antuitrin-S.

Blood cholesterols of the animals 6 to 9 and 13 to 25 months old were higher in females than in males.

Liver weight in relation to the total body weight was greater in females than in males in the untreated animals.

Animals 13 to 25 months old had higher fatty acids in blood and liver and higher liver phosphatides than the animals 6 to 9 months old.

Females injected with theelin showed the most marked increases in liver and blood fatty acids.

Antuitrin-S injections resulted in elevated blood, liver and muscle fatty acids in both males and females in many cases.

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THE ABSENCE OF CHANGES IN GASTRIC ACTIVITY AND OF GASTRO-INTESTINAL ULCERATION FOLLOWING HYPOTHALAMIC LESIONS IN THE MONKEY AND CAT

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Since Cushing's report in 1932 (1) of a series of patients with midbrain tumors and concurrent peptic ulcers, there has been a flood of reports, mostly of an experimental nature, of observations on the same general problem (2, 3, 4, 5). Practically all of these papers agree in that the authors were able to find and associate gastro-intestinal lesions with lesions of the brain stem or hypothalamus, and though the methods of producing the lesions were as numerous as the papers themselves, in practically all cases the lesions were made by "open" operation. In some cases the lesions were large ones, and because of the difficulty in reaching the hypothalamus and midbrain in small animals, the brains must have suffered a considerable amount of co-incidental trauma.

The purpose of this report is to set forth our experience with a series of cats and monkeys in which hypothalamic lesions were made under a uniform method of procedure, and in which pre-operative, post-operative, and post-mortem examinations were also uniformly made, in an attempt to discover what changes, if any, occurred in the activity of the stomach and intestines, and what histological evidence there might be of mucosal change in the gastro-intestinal tracts of these animals.

Forty cats and seven monkeys comprise our series of animals. Gastric activity and secretion were studied pre-operatively in five monkeys and three cats; two monkeys and thirty-seven cats were studied only by post-operative observation, which, for all animals in the series, consisted of constant examination of the stools and vomitus, of the general behaviour of the animals, of their diet, and of their nutritional state. In the pre-operative study of five monkeys and three cats gastric activity was determined by subcutaneous injections of 0.5 mgm. of histamine (ergamine acid phosphate) or by 50 cc. of 7 per cent alcohol introduced into the washed fasted stomach by means of a Rehfuss tube (without the olive). The contents of the stomach were removed thirty minutes after the histamine injection or after the introduction of the alcohol test meal, and it was then analyzed for free acid (using p-dimethylaminoazo-benzene), total

acid (using phenolphthalein), bile and blood (using the naked eye). Six control determinations were made over a period of one month, after which localized hypothalamic lesions were made. Weekly post-operative gastric analyses were performed over a period of two to three months, the animals being on the usual laboratory diet (fresh beef and milk for the cats, fresh fruit and vegetables for the monkeys). The stools were watched daily for any change in character or the occurrence of blood, and, likewise, a close watch was maintained to discover the presence and type of any vomitus, particularly whether it contained any blood.

In all animals the lesions were made by electro-coagulation with the aid of the Horsley-Clarke stereotaxic apparatus. Under light nembutal anesthesia (administered intravenously, 30 mgm. per kgm. weight of the animal) the head of the animal was surgically prepared, a button of bone one centimeter in diameter was removed from the vertex of the skull, and the apparatus was secured to the head. Without incising the dura mater, a monopolar electrode was passed through the dura down to the desired hypothalamic area, and the lesion then produced by the passage of a current of three milliamperes for from fifty-five to sixty-five seconds. Some lesions were made in the midline; others, bilaterally. Thus, in the entire series, eventually a block area of the hypothalamus between the mammillary bodies and the optic chiasm, as far laterally as 2.5 mm. from the midline, and from the under surface of the hypothalamus dorsalward 7 mm. was systematically explored by the production of lesions, the same lesion being made in some cases in several animals, so that the results from any one lesion could be checked against the results of the same lesion in another animal. In all of the monkeys and in a fourth of the cats the lesions were made entirely within the tuber cinereum in an effort to parallel the results of Hoff and Sheehan. In most cases the animals had returned to normal in thirty-six hours, and were back on their regular diet in forty-eight hours. In some of the cats a state of narcolepsy persisted for three to five days, but this invariably gave way to normalcy if the animals lived. Three cats died within seventy-two hours, never arousing from their post-operative stupor, and one cat died on the seventh day of a wide-spread cellulitis of the head. The monkeys were killed at varying intervals, from forty-two to one hundred ninety days post-operatively; the three cats that had had pre-operative gastric analyses were killed at the end of one hundred and two days; and the remaining thirty-seven cats were killed from twenty to forty days post-operatively, so that ample opportunity was afforded to study the effects of the lesions in the acute, subacute, and chronic stages.

It was found that there was no significant post-operative change in the gastric motility in any of the animals as evidenced by the emptying time

of the alcohol test meal. There was also no significant alteration in the free and total acidity, either in response to alcohol or to histamine, although one of the cats and two of the monkeys occasionally showed a higher acidity post-operatively than they had shown in any of the pre-operative control tests. None of the gastric samples of washings contained blood or bile, nor was blood present at any time in the stools of any of the animals. In no case did we discover any bloody vomitus, and the appetite was in most cases normal as soon as the animal regained full consciousness. In one monkey a profuse menstrual flow appeared on the third day after operation and persisted for four days. An occasional cat showed an increased thirst, and others showed a drowsiness characteristic of lesions in certain hypothalamic areas.

The brains of the animals were saved for verification of the lesions, and a careful post-mortem search was made of the gastro-intestinal tracts in an effort to discover some pathological change in the mucosa. In not one animal were we able to find any acute or chronic ulceration of the lower esophagus, stomach, or small bowel. In none was there any mucosal erosion which could be interpreted as anything but the usual post-mortem change, and, in general, the animals were in a good state of nutrition when sacrificed.

In some of the experimental work already reported, the lesions were made by incising the corpus callosum and then sucking out the floor of the third ventricle, or by retracting the temporal lobe and cutting the tuber area with a hook, or by nicking the posterior hypothalamus with a sharp hook, sometimes with the loss of cerebro-spinal fluid. They were in practically all cases made by the usual operative approach to the head in laboratory animals, and no doubt blood was in many cases introduced into the ventricles. There can be no doubt as to the presence of lesions in these animals, but in some cases it would seem that the lesion was too large for it to have any character as to specific location, and that unintentional operative trauma, with uncontrolled side actions, may well have resulted in a fair number of cases. Magoun, Ranson and Hetherington (6) have recently shown how widespread are the autonomic pathways leading down the brain stem from the hypothalamus, and their evidence gained with large lesions at various levels in the brain stem, together with our results here reported, indicate that mucosal change or disturbed physiology cannot be produced in the digestive tract of cats or monkeys by small, isolated, specifically located, experimental lesions in the hypothalamus.

SUMMARY

Small, localized lesions were made by the Horsley-Clarke apparatus in the hypothalamic area of seven monkeys and forty cats. Five monkeys

and three cats were extensively studied both pre- and post-operatively in an attempt to check gastro-intestinal activity and secretion in the normal with that of the lesion animal. All animals in the series were observed after operation for any signs of gastro-intestinal pathology, and, following autopsy at the end of from twenty to one-hundred ninety days, the brain lesions were verified and the gastro-intestinal tracts searched for evidence of ulceration or mucosal erosion. (Fig. 1.)

No bloody stools or vomitus were observed at any time in any of the animals; their nutritional state remained good; there were no significant changes in gastro-intestinal activity or secretion following the production of the lesions; autopsy did not reveal any mucosal changes in the gastro-intestinal tracts which could be ascribed to the lesions.

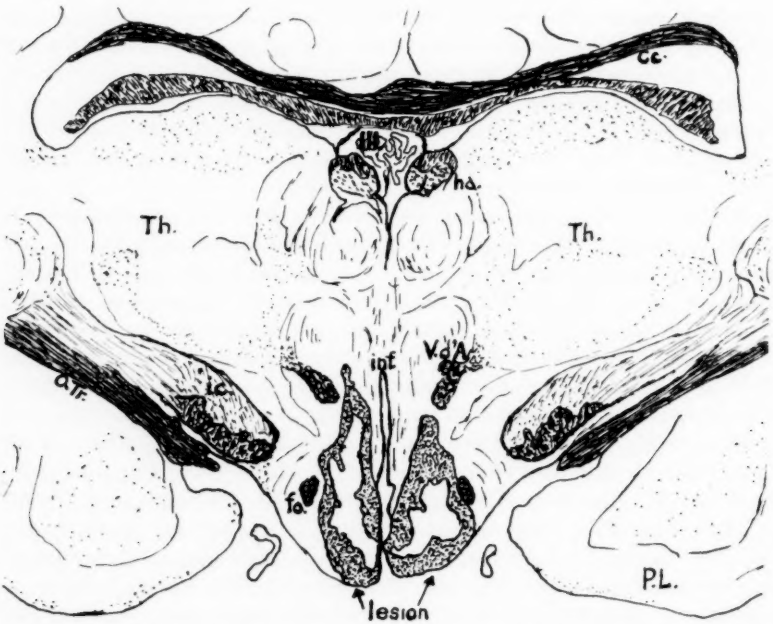

Fig. 1. This is a drawing of a portion of a cat's brain, made by means of an Edinger projection apparatus. It shows the hypothalamic area, with the lesion, in one of the animals used in this study, and it is typical of the lesions occupying the region of the infundibulum. The central portion of the lesion here shown has undergone a cystic degeneration. Magnification x 6¾. (Cc., corpus callosum; *III*, third ventricle; *Th.*, thalamus; *ha.*, habenula; *inf.*, infundibulum; *V. d'A.*, bundle of Vicq d'Azyr; *i. c.*, internal capsule; *fo.*, descending column of the fornix; *O. Tr.*, optic tract; *P. L.*, pyriform lobe.)

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A STUDY ON THE CONTROL OF LENGTH OF GESTATION IN THE RAT WITH NOTES ON MAINTENANCE AND TERMINATION OF GESTATION

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The factors governing the length of pregnancy in each mammalian species must emanate from the mother, the fetus or the placenta. Smith (1932) has demonstrated that the posterior lobe of the maternal hypophysis is not essential to the maintenance of gestation or to normal timing of labor in the rat. The anterior lobe is not required for the maintenance of pregnancy, although evidence bearing on its relation to the length of gestation in this species is conflicting (Selye, Collip and Thomson, 1932; Pencharz and Long, 1933). Ovariectomy is incompatible with normal pregnancy in the rat (Nelson and Haterius, 1930; Johnson and Challans, 1930), but Haterius (1936) has shown that removal of both ovaries and all fetuses in excess of one in pregnant rats does not interfere with the maintenance of the single fetus, even though delivery is not accomplished. Selye, Collip and Thomson (1935) removed the embryos from the uteri of gestating rats between the ninth and thirteenth days of pregnancy and observed that the remaining placentae were retained *in utero* "for a period of about eight to twelve days, at the end of which time they were aborted. . . ." Since this abortion took place about the time of the expected parturition the authors concluded that "the placenta determines the length of pregnancy."

This conclusion is open to two criticisms: 1, since all of the operations were performed about the middle of pregnancy and in every case the placentae were delivered "about" ten to twelve days later, the possibility arises that a placenta, in the absence of its fetal neighbor, requires about ten to twelve days to disengage itself from its uterine environment and is then expelled as a foreign body; 2, these investigators have failed to rule out maternal factors which might be operating to control the length of *in situ* maintenance of the placentae; i.e., even if the placentae were born precisely at the time of the expected parturition this may have been the result of purely maternal factors acting on the placentae, just as they would normally act on the undisturbed term fetus. Furthermore, Haterius (1936) indicates that in the absence of the ovaries (maternal factors),

the placentae remaining *in utero* after excision of the embryos were invariably retained at least two days past the date of parturition and were not delivered. These data, thus, seemed to bear out both of the above objections.

Effect of removal of embryos. Experiments were designed to test the effect of removal of embryos from the uteri of rats pregnant for varying lengths of time. Adult females were caged with adult males and were examined each morning for the vaginal plug and for spermatozoa in the vagina; the mated females were then considered to be in the middle of the first day of pregnancy. Of 10 control animals parturition took place in seven cases on day twenty-three, with a maximum variation of one day in the remaining three. The embryos alone were removed from 10 experimental animals between the fourteenth and twenty-first days of gestation. This removal was accomplished under ether anesthesia by preparing a purse-string suture through the ab-placental surface of each gestation chamber; a small incision was made in the center of the suture-ring, the embryo was gently expressed and the umbilical stalk was cut as near its placental attachment as possible. The purse-string suture was drawn tightly closed and knotted, invaginating the incised part of the uterus in the process. In every case the placentae were retained *in utero* at least until day twenty-two (when exploratory laparotomy was performed), and were delivered between this date and day twenty-three to twenty-four. It is thus established that regardless of the time of removal of the fetuses, the placentae remaining *in situ* are retained until term, at which time they are delivered.

The question then arose whether the placentae alone would respond to maternal labor as would the normal fetuses and placentae. Therefore, five mated rats were subjected to a removal of fetuses from one uterine horn only, on the 15th, 17th and 19th days of gestation. These animals retained the remaining placentae in the operated horn for the same length of time that the fetuses and placentae of the contralateral horn were maintained, as determined by laparotomy, and exploration on the day following the birth of the living young disclosed the absence of the placentae from the operated side. The placentae, then, in the local absence of the fetuses that they formerly nourished, are capable of responding to the normal birth mechanism. Inasmuch as Selye, Collip and Thomson (1935) have shown that the presence of placentae *in utero* is sufficient to maintain mammary gland, vagina, uterus and ovary in a typically pregnant state, the fetus is not needed for the maintenance of the maternal organism in a "gestating" condition. The length of time that the placentae remained *in utero*, in the present experiments, may thus be taken as a criterion of the length of pregnancy; therefore, it becomes justifiable to conclude that the fetus plays no essential rôle in governing the length of pregnancy in

the rat. This indicates the invalidity of those theories of labor which attribute the essential cause of its onset to fetal hormones, fetal metabolic and waste products acting on maternal nerve centers, and to an anaphylactic action of fetal blood. The essential stimulus to labor must hence reside in either the placenta or the mother.

Effect of bilateral ovariectomy and removal of embryos. Experiments were then formulated to rule out or determine the necessity of various maternal and placental factors. Selye *et al.* (1935) and Haterius (1936) have indicated that the placentae may survive after ovariectomy in pregnant rats; the former group sacrificed the animals several days before term, and the latter found the placentae, two days after the date of expected delivery, still *in situ*. Therefore, in attempting to determine the rôle of the ovaries, 15 rats were subjected to bilateral oöphorectomy (in which the entire ovarian capsule, oviduct and tubal end of the uterus were excised) and to complete removal of the embryos between the 15th and 19th days of gestation. In addition, to standardize these and the subsequent experiments, all but the three placentae nearest the cervical end of each uterine horn were removed wherever possible; this further obviated the possibility of mechanical retention of placentae due to involution of the uterus distal to the loci of their attachment. Of these 15 animals the majority aborted at least one of the placentae within three days following ovariectomy, 8 of the animals retained the majority of the placentae past 48 hours after ovariectomy, while only one-third of them retained placentae until term. Of this latter group two rats delivered all of their retained placentae at term, whereas the remaining three retained one or more placentae past term.

Normal pregnancy in the rat is invariably terminated by ovariectomy, usually within 48 hours. Nevertheless, while ovariectomy in the present experiments was followed in the majority of cases by abortion of at least one placenta, still the majority of the placentae were retained past 48 hours after the operation, and under the special conditions of the experiments of Haterius (1936) one fetus could be maintained after ovariectomy; it appears thus that in such special instances a quantitative need may determine the essentiality or non-essentiality of the ovary. It is justifiable to conclude that in these two special experiments the rôle of the ovaries in maintaining the placenta and in maintaining uterine quiescence is of subordinate importance. The ovary, however, has some rôle in maintaining multiple placentae, but this rôle is neither absolute nor consistent.

The rôle of the placenta. At this point, then, the part played by the ovaries in maintaining the normal pregnant rat in a gestating condition had not been exactly defined; hence they could not be excluded from being the agents controlling the length of pregnancy. However, experiments were devised to determine whether there exist other factors equally as

essential to the maintenance and control of length of gestation. The uteri of 5 rats, pregnant for 15 to 19 days, were incised and all embryos and placentae were removed; into the now-empty gestation chambers of each uterine horn were placed sterile paraffin pellets equal in size and shape to the excised embryo and placenta. A purse-string suture was made over each incision, drawn tight, and the animals were allowed to recover. Invariably, in 40–48 hours after the operation these pellets were aborted. However, in 10 animals the experiments were repeated with the exception that three or four placentae were allowed to remain *in situ* in one uterine horn, and paraffin pellets inserted in the same or opposite horn were not aborted; on the contrary, these pellets in the presence of functional placental tissue, in all 10 cases, were retained *in utero* until the day of expected parturition, at which time they were delivered.

It was necessary, then, to determine whether the *in utero* retention of the paraffin pellets until term could be regarded as a true criterion of the length of pregnancy in these animals. The contents of one uterine horn of five animals pregnant 15 to 18 days were replaced with paraffin pellets, the embryos and placentae in the opposite horn remaining undisturbed. On the 23rd and 24th days these animals went into labor and delivered living young as well as the paraffin pellets. The pellets are thus capable of responding to the normal birth mechanism. Inasmuch as it has been shown above that the length of *in utero* retention of the placentae is a criterion of the length of pregnancy, and since the paraffin pellets are retained only in the presence of placentae, the time that the pellets remain *in utero* may be used as a measure of the length of uterine quiescence characteristic of gestation. Therefore, the placenta is at least as essential as the ovary to the maintenance of normal pregnancy, and cannot be excluded from being the essential governor of its length. Indeed, inasmuch as the effect of ovariectomy on the maintenance of placentae in the present experiments has been so uncertain, and since removal of the placentae is followed by such a precise termination of pregnancy, it seems highly probable that the placenta, at least under the special conditions of these experiments, is not only the more important element in maintaining gestation, but also the ultimate controller of the length of gestation. However, since in no experiment have the placentae been separated from their uterine insertion without terminating pregnancy, this controlling mechanism, for the present, must be said to reside in the "placento-uterine complex" (i.e., the placenta and the uterus underlying it). It is still theoretically possible that some other maternal agency operates to terminate the influence of the placento-uterine complex and hence to terminate pregnancy, but there is now no evidence of the existence of such a factor.

Essential stimuli for the maintenance and probably for the termination of pregnancy must come, therefore, from the placenta, the uterus, or both.

As to the mechanism whereby the placento-uterine complex participates in this controlling scheme, three possibilities arise: 1, mechanical; 2, neural; 3, humoral. That it is not mechanical is made evident by the fact that the inert paraffin pellets in the above experiments were alone unable to prevent premature termination of pregnancy, although certainly they exerted as great mechanical effects on the uterus as did the placentae. Moreover, when pellets and placentae were left *in utero* their delivery was not accomplished until the day of expected parturition, despite the constancy of mechanical distention by the placentae and the larger paraffin pellets. The work of Kruieger and Offergeld (1907) in rabbits, guinea pigs and dogs, indicates that the uterus deprived of all its extrinsic nervous connections could nevertheless carry on pregnancy to a normal correctly-timed parturition. In an attempt to rule out the intrinsic nerves of the uterus as the route by which the placento-uterine complex exerts its effect, 3 rats pregnant for 15 to 19 days were treated as follows: all embryos were removed from one uterine horn, the placentae being left *in situ*; the contents of the contralateral horn were completely removed and replaced with paraffin pellets. The horn containing the placentae was then completely severed from its attachment to the cervix and to the horn containing the pellets, so that all communicating intrinsic nerve pathways were interrupted. Despite these maneuvers the placento-uterine complex was still able to exhibit its usual control, for the pellets in the opposite horn were retained until the day of expected term, and were then delivered. The placentae, retained because of the ligature, were nonetheless disengaged and movable at autopsy. It is concluded, thus, that the essential cause of onset of labor is probably a hormonal mechanism, and that the rôle played by the placento-uterine complex, both in maintaining the uterine quiescence characteristic of gestation and in controlling the length of gestation, is primarily on a humoral basis. This is direct evidence of the endocrine nature of the placento-uterine complex. It is also concluded that the placenta, whether it acts directly or by way of the uterus or other maternal system, is essential to the maintenance of the uterine quiescence of pregnancy.

Discussion. The fact, indicated by Selye, Collip and Thomson (1935) and established in the present communication, that in the rat the fetus is not essential to the maintenance of gestation and its uterine quiescence, may be subject to some species difference. In the mouse, Newton (1935) ruptured the amniotic sacs by firm external pressure, thus killing the fetuses. The placentae remained *in situ* until term and were delivered. Inasmuch as this worker mentions that these fetuses disintegrated, the possibility that they liberated some progestational substance has not been excluded. The data of Hammond (1917), Klein (1934) and Nelson (1934) allow no specific conclusions concerning the fate of placentae after embryo-removal in the rabbit and guinea pig.

Mechanism of maintenance of pregnancy. The experiments of Fraenkel (1910), Corner and Allen (1929) and Allen and Corner (1930) show that corpus luteum hormone is essential to the maintenance of pregnancy in the rabbit. The work of Selye, Collip and Thomson (1935) and Haterius (1936), discussed above, indicates that this pregnancy-maintaining principle may be elaborated by the placenta, although the possibility of some uterine contribution has not been ruled out; indeed, the reports of Duval (1891) and Selye *et al.* (1935) point to a possible endocrine function by uterine cells. In the light of these data and of the present experiments it is apparent that the placento-uterine complex contributes to the maintenance of gestation by secreting progesterone or a related substance. This finding is a probable explanation for the maintenance of pregnancy after ovariectomy in the human (Pratt, 1927), guinea pig (Nelson, 1934) and mare (Hart and Cole, 1934), and is supported by the successful extraction of progesterone from placentae by Adler, Fremery and Tausk (1934) and by van Lankeren (1935).

Mechanism of termination of pregnancy. The termination of gestation can only be the result of 1, a diminution or cessation of secretion of some inhibitory hormone secreted during pregnancy, or 2, the initiation of secretion of effective amounts of some stimulating principle at term, or possibly 3, both. Snyder (1934) and Koff and Davis (1937) present strong evidence that labor is due to a diminution of circulating progesterone. Since Allen and Corner (1930) have found that normally-timed parturition occurred in castrate pregnant rabbits receiving constant amounts of progesterone, the diminution of *extra-ovarian secretion* of progesterone is probably important in causing labor. The possibility of parturition resulting from diminution of secretion of gonadotropic substance must be considered. Finally, there is evidence that estrogens, secreted near term in active form and quantity, may be a stimulus to parturition (cf. Ascheim and Zondek, 1928; Cohen, Marrian and Watson, 1935; Reynolds, 1935).

It is proposed, therefore, that the placento-uterine complex probably initiates labor by contributing to one of these possible mechanisms, although the exact nature of its action remains to be determined. For the present the principal conclusion is that in the rat, and probably in higher forms, the placento-uterine complex has an essential endocrine rôle which is directly concerned with the maintenance and probably the control of length of gestation.

SUMMARY AND CONCLUSIONS

In the rat:

1. Regardless of the time of pregnancy at which surgical removal of embryos alone from the uterine horns is performed, the placentae remaining *in situ* are retained until the date of expected parturition, at which

time they are delivered. 2. These remaining placentae are capable of responding to the normal birth mechanism. 3. The length of time these placentae remain *in utero* may be taken as a criterion of the duration of pregnancy. 4. Paraffin pellets replacing embryo and placenta *in utero* are retained until full term providing functional placentae are present in the same or contralateral horn. 5. The length of retention of these pellets *in utero* is a criterion of the length of pregnancy of the maternal organism. 6. The fetus is excluded from being the essential regulator of the length of pregnancy, and cannot be, either mechanically or chemically, the cause of onset of labor. 7. The ovaries have a rôle in maintaining the placenta, but this rôle is neither absolute nor consistent. 8. The presence of the placenta is essential for the maintenance of the uterine quiescence characteristic of pregnancy. Whether this influence is direct or indirect is not known. 9. The "placento-uterine complex", under the special experimental conditions described, is the essential factor in maintaining gestation and probably in controlling its length. It is believed that this complex has similar functions in the normal gestating rat and probably in higher animals. 10. These functions of the placento-uterine complex are on a humoral basis; this is, therefore, direct evidence of the endocrine rôle of this complex. 11. The placento-uterine complex is believed to secrete progesterone or a similarly acting principle. 12. The importance of the placento-uterine complex in the birth mechanism and the possible mechanisms of its action are discussed.

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THE SENSITIZATION OF THE SUPERIOR CERVICAL GANGLION TO NERVE IMPULSES BY PARTIAL DENERVATION

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Cannon and Rosenblueth (1936) have recently demonstrated that the chronically denervated superior cervical ganglion gives greater responses than the normal ganglion to intravenous injections or local applications of acetylcholine, the chemical mediator of nerve impulses in sympathetic ganglia (Kibjakow, 1933; Feldberg and Gaddum, 1934). That the denervated nictitating membrane of the cat is hypersensitive to the first nerve impulses reaching it after regrowth of the nerve fibers has already been suggested (Simeone, 1937). It was considered of interest to test whether the partially denervated ganglion would similarly become sensitized to nerve impulses, since the demonstration of sensitization within neuronal systems might have broad implications.

METHOD. Adult cats were used throughout these experiments. The right or left superior cervical ganglion was partially denervated by severing the rami communicantes from the first and second thoracic nerves (T-1 and T-2) to the stellate ganglion within the chest. No attempt was made to separate white and gray rami. It was expected that this procedure would lead to degeneration of 50 per cent or more of the preganglionic fibers supplying the nictitating membrane (cf. Langley, 1900). All operations were done with aseptic precautions, ether anesthesia and artificial positive-pressure respiration.

The experiments were done 3 to 6 weeks later under dial anesthesia (Ciba, 0.75 cc. per kgm. intraperitoneally). With the animal on artificial respiration, the thoracic sympathetic trunk was cut or crushed below its communication with the fourth thoracic nerve on both sides. The sympathetic chain below T-2 was placed on shielded electrodes to include and stimulate the fibers from T-3 and T-4. The electrodes were placed in corresponding positions on the two sides. The rami from T-1 and T-2 were cut acutely on the normal side. Dry cotton was packed around the electrodes and this was inspected from time to time during the course of the experiment. It was often necessary to change the cotton packing because of excess moisture, and this was found more frequently on the side

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operated upon than on the control side. The stimuli used were either induction shocks from a Harvard coil with 6 volts in the primary circuit or condenser discharges. The stimulating circuits were kept entirely separate on the two sides.

In some experiments, the stimulating electrodes were placed on the cervical sympathetic trunk. Here, of course, the number of fibers stimulated on the normal side was greater than that stimulated on the partially denervated side.

The responses of the superior cervical ganglia to electrical stimulation of the preganglionic fibers were studied in two ways—mechanically, using the contractions of the corresponding nictitating membranes, and electrically, recording the action potentials of the postganglionic fibers. Kymographic records were made of the isotonic contractions of both nictitating membranes recording simultaneously with 19-fold magnification and 4.0 grams tension. The eyelids were not cut as a rule and seldom interfered with the recording. Simultaneous tracings were also made of the contractions of the nictitating membranes in response to standard doses of adrenalin injected into the femoral vein to evaluate the degree of sensitization of the partially decentralized membrane to adrenine (and sympathin). The action potentials of the postganglionic fibers and nictitating membranes were recorded by means of a 5-stage capacity-coupled amplifier and cathode-ray oscillograph.

Results. A. *Responses of the normal and partially denervated superior cervical ganglia to stimulation of the preganglionic fibers in the rami from T-3 and T-4.* Figure 1A shows the responses of the right (upper record) and left (lower record) nictitating membranes. The right superior cervical ganglion had been partially denervated 3 weeks previously. The rami from T-1 and T-2 were cut acutely on the left side and maximal condenser discharges were applied to the fibers from T-3 and T-4 on both sides. The difference in the responses can in part be attributed to the slight degree of increased sensitivity of the nictitating membrane to adrenine (and sympathin). This difference in sensitivity, however, was only minimal, as demonstrated by the corresponding responses to adrenalin (10γ, fig. 1B). Furthermore, stimulation of the postganglionic fibers after the ganglia were crushed yielded practically equal responses on both sides. It may be concluded that the larger response of the right membrane in figure 1A is due to a sensitization of the ganglion by denervation.

Similar results were obtained in the seven animals tested. Usually the response on the denervated side was at least double that on the normal side (fig. 1). The extreme instance is illustrated in figure 2. It should be emphasized that since maximal shocks were used in all experiments any spread of the stimuli would favor the normal side, as some of the cut fibers from T-2 and T-1 might thereby be stimulated.

The maximal responses of the two sides to tetanizing currents were usually equal and sometimes the response of the non-sensitized side was actually greater, eliminating the possibility that the greater response to single shocks on the sensitized side might be due to differences in the recording devices or a greater capacity for contraction in one membrane as compared with the other.

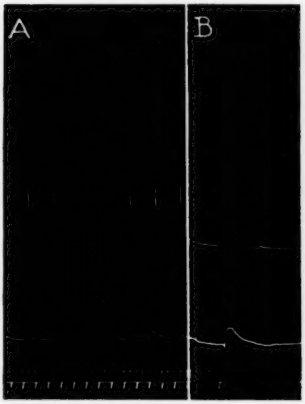

Fig. 1

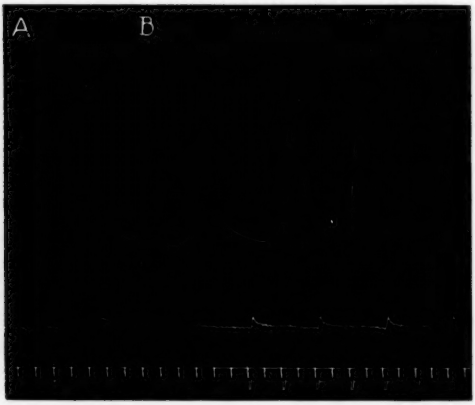

Fig. 2

Fig. 1. Dial (0.8 cc. per kgm. intraperitoneally). Right superior cervical ganglion partially denervated 3 weeks previously by cutting rami T-1 and T-2. Both thoracic chains crushed below T-4. Fibers from T-1 and T-2 cut acutely on left. Electrodes on thoracic sympathetic chain on both sides, including fibers from T-3 and T-4. Upper tracing, right, and lower tracing, left nictitating membrane.

A. Responses to groups of 4 maximal condenser discharges at a rate of 1 per sec. Time signal: 30 sec.

B. Response to adrenalin (10γ) intravenously.

Fig. 2. Preparation and records as in figure 1.

A. Responses to adrenalin (2γ).

B. Responses to groups of condenser discharges applied alternately to the two sides at the signals.

B. *Responses to stimulation of the normal and partially degenerated cervical sympathetic trunks.* In one of the animals partial preganglionic denervation had produced no demonstrable increase in sensitivity of the corresponding nictitating membrane to small doses of adrenalin (fig. 3A). Comparable maximal stimulation of the fibers from T-3 and T-4 on the two sides, however, led to a contraction of the nictitating membrane on the denervated side at least twice that of the membrane on the normal side (fig. 3B). Figure 3C shows the responses of the right nictitating membrane (upper record) and left membrane (lower record) to maximal stimu-

lation of the cervical sympathetics in the same animal. It will be seen that the increased sensitivity of the right ganglion is not quite sufficient to compensate for the larger number of fibers stimulated on the left side. In other experiments slightly greater responses were obtained on the partially denervated side, in spite of the probable presence of less than half as many fibers on this side as on the control side. The difference in the responses in these cases, however, was usually small.

In three experiments the contractions of the nictitating membrane on preganglionic stimulation of the partially denervated side were compared with those on stimulation of the postganglionic fibers after crushing the superior cervical ganglion. The responses to similar stimuli, single shocks or repetitive, were found to be practically the same.

C. *The post-tetanic augmentation of the effects of stimuli to the partially denervated superior cervical ganglion.* Cannon and Rosenblueth (1937)

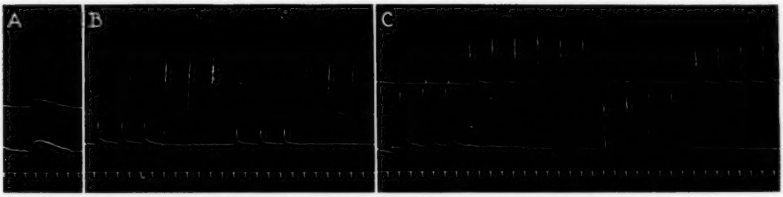

Fig. 3. Preparation and records as in figure 1.

A. Responses to adrenalin (5γ) intravenously.

B. Responses to groups of 4 maximal condenser discharges in 3 seconds applied to fibers from T-3 and T-4, alternately on the two sides.

C. Responses to same type of stimulation applied to cervical sympathetic trunks in the neck after crushing them centrally.

demonstrated the increased efficacy of maximal single preganglionic shocks to the superior cervical ganglion after faradization. This effect was confirmed during the course of these experiments for both the partially denervated ganglion (fig. 4) and the normal. In the former the post-tetanic effects may last a few minutes longer (1–4) than on the control side. That the effect is exerted through the ganglion is shown by its disappearance after crushing the superior cervical ganglion and stimulation of the postganglionic fibers.

D. *The electrical responses of the postganglionic fibers.* A comparison of the action potentials of the postganglionic fibers of normal and partially denervated ganglia after stimulation of the corresponding cervical sympathetic trunks showed no marked difference. Although the records of the partially denervated side suggested probable repetitive discharges of the postganglionic neurons in response to single preganglionic volleys, the

evidence in this respect was not conclusive. The maximal spike potentials on the chronically denervated side were approximately of the same magnitude as those on the normal side in spite of the fact that fewer preganglionic fibers were stimulated on the partially denervated side.

Discussion. The evidence for an increased sensitivity of the superior cervical ganglion to preganglionic nerve impulses after partial denervation is two-fold. *a*. Stimulation of an approximately equal number of preganglionic fibers on the two sides gives a much greater response from the partially denervated ganglion prepared chronically than from the similarly but acutely denervated ganglion (figs. 1 and 2). *b*. Stimulation of the preganglionic fibers in the neck leads to nearly equal responses on the

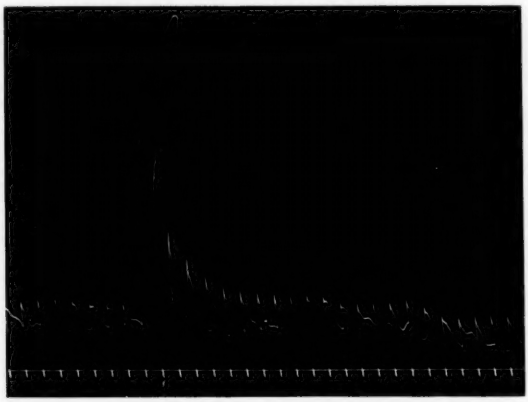

Fig. 4. Dial (0.8 cc. per kgm. intraperitoneally). Right superior cervical ganglion partially denervated 5 weeks previously. Electrodes on right preganglionic trunk, cut centrally. Responses (except that at signal) to groups of 4 maximal condenser discharges in 2 seconds. At signal, a 1-minute tetanus was applied through a pair of electrodes placed caudad. Time signal: 60 sec.

two sides even though the partially denervated side has probably less than half as many preganglionic fibers for the membrane as the normal side. The increased effects on the chronically denervated side are, no doubt, exaggerated by the slight peripheral sensitization of the nictitating membrane to adrenine (and sympathin). That this effect is of very little moment, however, has been pointed out above (figs. 1 and 2).

The increased responses of the denervated ganglion to impinging nerve impulses could be due either to discharges from a greater number of postganglionic elements than are normally activated by a given number of preganglionic fibers (a spatial effect), or to repetitive discharges from the same number of postganglionic neurons as on the control side where repeti-

tion is improbable (a temporal effect); or finally to both the spatial and temporal modes of increment.

That repetitive discharges of the ganglionic elements to single preganglionic volleys may appear in certain experimental conditions is probable from evidence presented by Cannon and Rosenblueth (1937). It is quite conceivable, therefore, that temporal effects may have occurred in the present experiments. Indeed, Brücke's (1937) report of a decreased concentration of cholinesterase in denervated superior cervical ganglia suggests the possibility of a persistence of the acetylcholine liberated by the nerve impulses and therefore a repetitive activation of the nerve cells in the ganglion.

A spatial spread of the sphere of action of a limited number of preganglionic fibers is likewise readily conceived since Eccles' (1935) demonstration of subliminal fringes of neurons in the ganglion—i.e., of postganglionic elements subliminally excited in normal conditions by submaximal preganglionic nerve volleys. It is possible that partial denervation may render such subliminal excitation effective, so that a larger number of nerve cells would be activated by a given fraction of the preganglionic fibers.

The present data (sections A, B and D) do not allow any definite conclusions with regard to the spatial or temporal nature of the increased responses of the partially denervated ganglion. The similar magnitude of the spike potentials of the postganglionic fibers upon stimulation of all the preganglionic fibers on both sides (section D) supports the inference of a spatial increment on the denervated side. On the other hand, slight asynchronous after-discharges may well have occurred in the electric responses recorded.

The increased sensitivity of partially denervated ganglia to preganglionic nerve impulses has an important bearing on the theories concerning the problem of synaptic transmission. The electrical theory of synaptic activation does not account for repetitive discharges of the neurons, if such occur. Even if the effects were entirely spatial, electric activation of a larger number of nerve elements by the nerve impulses could occur only if the electrical threshold of these elements were lowered by the denervation. The determination of the electrical excitability of normally innervated ganglionic neurons for comparison with that of denervated neurons is difficult. It is impossible, with the techniques available, to determine accurately the excitability of the normal nerve-cell body at the ganglion with the assurance that the preganglionic nerve endings are not activated. Nevertheless, unpublished observations (Simeone and Acheson) on the excitability of normal and denervated ganglia in the cat do not support the concept of a decreased electrical threshold of the denervated ganglion. For comparable responses of the nictitating membrane ($\frac{1}{3}$ the maximal response), the electrical threshold of the denervated

ganglion is almost invariably greater than that of the normal ganglion with condenser discharges of capacities varying between 1.0 and 0.03 microfarads. It is safe to conclude, therefore, that if the effect is spatial it probably is not explained by electrical activation of the previously subliminal fringe.

The electrical theory of synaptic transmission, then, does not appear to account satisfactorily for the increased sensitivity of the partially denervated ganglion to nerve impulses. The chemical theory, however, readily covers all aspects of the phenomenon. Temporal increments would be caused by the persistence of acetylcholine in the absence of sufficient cholinesterase for its rapid destruction (cf. Brücke, 1937); spatial increments would result from a lowered threshold of the neurons to the chemical mediator (cf. Cannon and Rosenblueth, 1936).

SUMMARY

Partial denervation of the superior cervical ganglion renders it hypersensitive to preganglionic nerve impulses (figs. 1, 2 and 3).

Possible mechanisms for the increased sensitivity of the partially denervated ganglion are discussed (p. 99).

The significance of the phenomenon in relation to theories of synaptic transmission of nerve impulses is considered (p. 99).

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INFLUENCE OF ADRENALECTOMY UPON KETOLYTIC ACTIVITY

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The removal of the adrenal glands reduces, or abolishes, the ketosis of fasting (1), pancreatectomy (2), phloridzinization (3), pregnancy (4), and following the administration of ketogenic anterior pituitary extracts (1) when it is measured by the ketonuria. Adrenalectomy may influence the ketosis in these conditions either by reducing the rate at which the ketone bodies are produced (*antiketogenesis*) or by increasing their rate of oxidation (*ketolysis*), or both. Experiments have been carried out designed to examine the ketolytic effect of adrenalectomy. A ketosis was produced (5) by feeding diacetic acid or beta-hydroxybutyric acid to rats. The extent of the ketone excretion then varied inversely as the percentage oxidation of these exogenous ketones.

EXPERIMENTAL. The adrenals were removed as usual (6) under ether anesthesia in a manner which has been used for several thousand rats. They recover from the immediate effects of the operation, which requires about two minutes when systematized, within five to ten minutes after ether administration has been discontinued. Urine collections and fatty acid administration were commenced at once. The reason for this is that any period allowed for further recovery from the operation per se (1) tends to make the rats become more susceptible to the necessary fasting and to the toxicity of the fatty acids. Of even more importance are the changes in other ductless glands following adrenalectomy, for we wished to obtain data influenced primarily by the removal of the adrenals.

The rats were kept in individual cages and supplied water or 0.9 per cent NaCl (expts. 1 and 2) *ad lib.* Urine was collected under light mineral oil and the total ketone bodies were determined on daily specimens by Van Slyke's method (7). In some experiments total urine nitrogen was determined by the macro-Kjeldahl method. The fluid administered by stomach tube, plus water the rats drank, was sufficient to produce good urine volumes (5–12 cc. per rat per day) in the adrenalectomized as well as the control rats. The rats were given one of their daily doses by stomach tube just before urine collections were made, and the slight struggle incident to this procedure generally made them empty their bladder completely.

TABLE 1*

EXPERIMENT NUMBER	GROUP	NUMBER OF RATS	SEX	BODY WEIGHT	BODY SURFACE	URINE EXCRETION IN MGM. PER 100 SQ. CM. BODY SURFACE PER DAY							
						Ketone bodies				Nitrogen			
						Days							
						1	2	3	4	1	2	3	4
Sodium aceto-acetate fed													
				gm.	*sq. cm.*								
1	C	4	♂	258	459	11	58						
	Ad	4	♂	272	475	12	29						
	C	4	♀	184	367	24	65						
	Ad	4	♀	197	385	13	29						
2	C	3	♂	253	453	31	10	35					
	Ad	3	♂	252	451	15	6	19					
3	C	3	♂	280	485	3	26	23	18				
	Ad	3	♂	284	487	1	16	11	2				
	C	3	♀	213	405	11	35	41	36				
	Ad	3	♀	220	413	2	12	15	2				
4	C	5	♂	281	486	19	25	23	24	35	27	23	19
	Ad	5	♂	290	496	11	6	4	5	24	21	22	15
	C	5	♀	187	372	23	29	37	44	38	27	25	20
	Ad	5	♀	197	385	14	10	6	3	24	25	24	19
Sodium β-hydroxybutyrate fed													
5	C	4	♂	314	520	18	29			43	29		
	Ad	4	♂	325	529	13	24			42	20		
	C	4	♀	205	395	21	36			40	35		
	Ad	4	♀	209	399	18	22			41	23		
6	C	3	♂	260	462	10	41	40		36	27	31	
	Ad	3	♂	258	459	8	17	37		27	21	19	
	C	3	♀	171	349	14	42			35	33		
	Ad	3	♀	174	353	11	29			25	17		
7	C	5	♂	203	392	13	48	65	74	25	19	24	24
	Ad	5	♂	205	391	8	20	16	22	24	20	23	18

* Fasting periods preceding operation varied from 0 hour in experiments 2, 5, 6, and 7 to 24 hours in experiments 1, 3, and 4.

Sham operations were performed on the control rats in experiments 1, 2, and 7. In the rest the controls were etherized but not operated upon.

The dose of 5 per cent diacetic acid fed each day in the form of the sodium salt was 100 mgm. per 100 sq. cm. body surface with the exceptions that the rats in experiment 2 were given 200 mgm. the first day and none on the second day, and those in experiment 3 were given only 50 mgm. per 100 sq. cm. body surface on the second day. The sodium beta-hydroxybutyrate was fed in experiment 5 as a 10 per cent solution in a dosage of 200 mgm., and in experiments 6 and 7 as a 7.5 per cent solution in a dosage of 150 mgm. per unit of body surface.

Sodium aceto-acetate was prepared from the ethyl ester (Eastman) and the sodium salt of racemic beta-hydroxybutyric acid purchased from the British Drug Houses. Except in experiment 6 the solutions, which were fed by stomach tube, were of such strength that each of the two daily doses was given on the basis of 1 cc. per 100 sq. cm. body surface. Experiments showed that diacetic acid and beta-hydroxybutyric acid have a very slightly reduced absorption rate in adrenalectomized rats. This is of no consequence in so far as the results presented here are concerned, for the 24 hour periods allow ample time for complete absorption, and any possible unabsorbed portion would show up in the collection the following day.

Results. Average results are presented in table 1. There is considerable variation in the figures for individual animals, but not such as to justify detailing them. Besides the dose of the ketone body fed and the factor (adrenalectomy) which concerns us here, there are numerous factors which may influence the extent of the experimental ketosis. Their influence on our data is nullified by the simultaneous collection of data from a comparable control animal for every adrenalectomized rat which was used. There are 22 pairs of group average observations in male rats and 14 pairs in female rats showing the influence of removing the adrenals on the degree of ketonuria. All except one pair (expt. 1, male, 1st day) show that after adrenalectomy the ketosis as measured in this manner and due to diacetic acid or beta-hydroxybutyric acid feeding is reduced. From these data we may conclude that in the absence of the adrenals there is a small but consistent increase in the percentage of either diacetic or beta-hydroxybutyric acid destroyed by oxidation or other metabolic processes.

Discussion. Although an increased ketolytic activity may account in some degree for the reduction in ketosis in the various conditions listed in the introduction to this note it is more probable that the antiketogenic action of adrenalectomy is responsible. The increased ketolytic activity of the organism which we have demonstrated here is none the less important. The fasting adrenalectomized animal has a low blood sugar (8), an almost complete disappearance of liver glycogen (8) and a deficient formation of glucose from lactic acid (9) and protein sources (3). It is possible that with this dearth of readily oxidizable carbohydrate upon which the organism is ordinarily so dependent that it turns to the ketone bodies when they are available for quick energy yielding substances. It is interesting in this connection that ketone bodies are always produced under those circumstances when there is an absolute lack of glucose or a relative deficiency of oxidizable glucose available for the body economy. It is hoped that a new blood ketone method (10) which has proved suitable for the study of arterial-venous differences (11) may make it possible to

determine the purposefulness of a ketosis in supplying oxidizable fuel of the order of glucose when the latter is unavailable.

SUMMARY

The ketonuria produced by feeding the sodium salts of diacetic or beta-hydroxybutyric acid to fasting rats is significantly reduced by adrenalectomy. This leads to the conclusion that this procedure increases the ketolytic activity of the organism.

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ADDENDUM—We are well aware that there is a lack of agreement as to whether or not the method (5) used here measures ketolytic activity. This method has been used for studying the relative ketolytic activity of glucose and some related compounds (Shapiro, I., J. Biol. Chem. **108:** 373, 1935), and it demonstrated marked ketolytic action by glucose, but the evidence is apparently not acceptable to some authors (Friedemann, T. E., J. Biol. Chem., **116:** 133, 1936; Mirsky, I. A. and R. H. Broh-Kahn, This Journal **119:** 734, 1937), who have ignored it. Although it is possible on theoretical grounds to explain the action as antiketogenic rather than ketolytic the current evidence does not favor such a view.

FACTORS INFLUENCING THE SERUM BICARBONATE CONCENTRATION OF THE DOG DURING TREADMILL EXERCISE[1]

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In an earlier report (1) it was shown that the serum of the dog which has exercised strenuously for an hour or more often shows a greater decrease in bicarbonate concentration than can be attributed to the usual effects of overventilation or to the entrance of lactic acid into the blood stream. From the standpoint of acid-base balance, such a decrease in bicarbonate concentration can be accounted for either by the loss of fixed base from the serum or by the increase of acid ions other than bicarbonate.

In order to determine the factors involved in the decrease of bicarbonate concentration, a study has been made of the changes in the acid-base system of the serum which occur as a result of exercise. This study has included determinations of serum water, protein, chloride, phosphate and total fixed base in addition to those of pH, total carbon dioxide and lactate. A description of the experiments and an account of the changes which occur in the concentrations of serum protein, fixed base and chloride during exercise and recovery have been given in a previous paper (2). The present report deals with the effects of strenuous, long-continued exercise upon the electrolyte pattern of the serum of the dog and attempts to explain the decrease in bicarbonate concentration as a compensation for a number of electrolyte changes.

METHODS. The methods have been described in previous reports (2, 3). The gasometric method of Avery and Hastings (4) for determining serum lactate has been modified slightly by using the Somogyi (5) reagents, copper sulfate and sodium tungstate, as the first precipitating agent, and extending the reaction time with permanganate to 6 minutes.

DISCUSSION OF RESULTS. The average results for those experiments in which samples of blood were drawn both at exhaustion and after 2 hours'

[1] This work has been conducted under a grant from the Douglas Smith Foundation at the University of Chicago.

[2] The authors are indebted to Miss Helen Oldham and Miss Elizabeth Riddle for assistance in the experimental work. They wish to express to Professor A. Baird Hastings their deep appreciation of his valuable criticism and suggestions.

TABLE 1

*Average changes in the concentration of various components of the serum as a result of exercise and recovery**

		DOG 3	DOG 6	DOG 5	
				(a)	(b)
Number of experiments run		4	5	5	11
Time to exhaustion, minutes		128	133	132	302
pH	Initial	7.37	7.41	7.40	7.41
	Exhaustion	7.49	7.52	7.40	7.39
	Recovery	7.34	7.39	7.37	7.37
Protein, grams per kilogram H_2O	Initial	63.1	63.9	66.8	66.8
	Exhaustion	64.2	67.5	67.3	70.2
	Recovery	61.8	63.7	67.8	70.8
Bicarbonate, m.-eq. per kilogram H_2O	Initial	22.7	24.5	23.4	24.0
	Exhaustion	13.7	18.7	20.2	19.6
	Recovery	19.7	22.5	22.1	20.7
Lactate, m.-eq. per kilogram H_2O	Initial	2.7	3.0	3.1	3.5
	Exhaustion	5.0	3.6	2.9	2.9
	Recovery	2.1	2.2	2.6	2.5
Phosphate (×1.8), m.-eq. per kilogram H_2O	Initial	4.05	2.51	3.00	2.54
	Exhaustion	2.65	1.72	2.26	3.27
	Recovery	4.40	2.71	3.82	4.32
Chloride, m.-eq. per kilogram H_2O	Initial	120.4	117.6	118.7	119.0
	Exhaustion	129.8	128.4	126.5	134.5
	Recovery	128.3	129.3	126.2	134.4
Sum of determined anions, chloride, phosphate, lactate, bicarbonate and proteinate, m.-eq. per kilogram H_2O	Initial	164.9	163.4	164.8	165.5
	Exhaustion	167.4	169.7	168.7	177.4
	Recovery	169.3	172.0	171.2	178.9
Total fixed base, m.-eq. per kilogram H_2O	Initial	168.5	169.1	168.0	170.8
	Exhaustion	174.3	179.0	175.2	186.5
	Recovery	174.8	178.7	174.9	185.5
Anion deficit, m.-eq. per kilogram H_2O	Initial	3.6	5.7	3.2	5.3
	Exhaustion	6.9	9.3	6.5	9.1
	Recovery	5.5	6.7	3.7	6.6

* Two hours of rest following exhaustion were allowed for recovery in each case.

recovery are shown in table 1. This table includes data for 3 dogs. For purposes of comparison, the experiments with dog 5 have been divided into two sections: section a includes 5 experiments in which the dog ran about 2 hours at the rate of 6 miles per hour, and section b 11 experiments in which the dog ran approximately 5 hours at a rate of 5 miles per hour. Dogs 3 and 6 ran at the slower rate of 5 miles per hour. Their running capacity was definitely less than that of dog 5, since they could not run much longer than two hours at this rate. In all of these experiments the treadmill was set at a 15 per cent incline and the room temperature remained fairly constant between 20° and 22°.

At exhaustion, the sera of dogs 3 and 6 show the usual results of overventilation, a reduced bicarbonate concentration and CO_2 tension and a rise of pH; the serum of dog 5 also shows a reduced bicarbonate concentration but, in the majority of runs, a slight reduction in CO_2 tension and no rise in pH. The serum lactate concentration rises slightly in the case of dogs 3 and 6, but falls slightly in the case of dog 5. The serum phosphate concentration decreases with exercise in all experiments except those in which dog 5 runs over four hours. Serum chloride and total fixed base increase in concentration in every case.

A comparison of the total fixed base concentration found by analysis with the sum of all of the anions determined, chloride, phosphate, lactate, bicarbonate and proteinate,[3] shows a difference of several milli-equivalents. Since the fixed base concentration is the larger, this difference may be termed the anion deficit. In the majority of experiments the anion deficit of the serum increases with exercise by approximately 3 to 5 milli-equivalents. Apparently anions other than those determined increase in the serum during exercise.

At present no adequate suggestion as to the identity of these undetermined anions can be offered. The possibility of sulfates and of acetoacetic acid or β-hydroxybutyric acid has been investigated. Each of these increases in concentration with exercise, but the increase is not more than a small fraction of one milli-equivalent. Johnson and Edwards (7) have shown that pyruvate increases in the blood during exercise, but again the increase amounts to a small fraction of one milli-equivalent. It would seem that the increase in the anion deficit with exercise is due to the collective increase of a large number of anions whose individual concentrations in the serum are small.

Figure 1 shows the results of following the changes in the composition of the serum during the course of a typical experiment with dog 5. In the diagram the changes in the various components of the serum are plotted

[3] Proteinate has been calculated from the equation of Van Slyke, Hastings, Hiller and Sendroy (6), milli-equivalents of base bound per gram of total serum protein nitrogen $= 0.66\ (pH_s - 5.08)$.

against the length of time the dog had run when the sample was taken. The chart shows that a rise in pH and in lactate occurs early in the experiment, but as the dog continues to run the pH and lactate return to the initial levels. The bicarbonate concentration, on the other hand, falls abruptly at first, then rises slightly, but falls again as running continues. A similar bicarbonate curve was found by Hastings (8) in the case of a dog

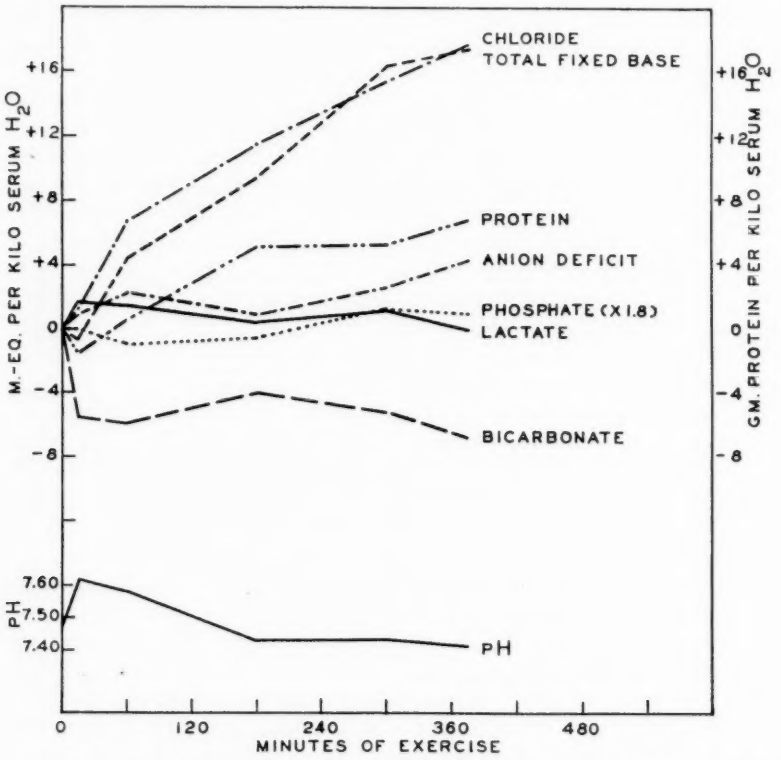

Fig. 1. Changes in concentrations of various serum components of dog 5 during exercise. A typical experiment. Changes are expressed in terms of milli-equivalents per kilo serum water.

who maintained a high rate of running for approximately nine hours. The phosphate concentration falls at first, but rises above the initial level as the dog continues to run. An anion deficit appears even in the first fifteen minutes of running.

In a preceding report (2) it has been shown that the body fluids become more concentrated during long-continued exercise as a result of loss of

body water, and that the extracellular fluids are affected to a greater extent than the intracellular fluids. The effect of such loss should be considered in any comparison of the changes occurring in the various components of the serum during exercise. For this purpose the increase in the serum chloride concentration in the two hour recovery sample over the initial value has been taken as a measure of the dehydration of the serum and other extracellular fluids, because it has been shown that the increase in the serum chloride concentration parallels fairly closely the loss of water from that system and the loss of chloride by way of the kidneys is markedly reduced during exercise (2). By choosing the recovery sample rather than that at exhaustion, any effect of pH shift on the serum chloride concentration has been virtually eliminated since the pH has returned approximately to the initial value by the time the recovery sample is drawn.

By using the increase in serum chloride as a measure of serum dehydration it has been possible to determine the changes in serum electrolytes which are due to factors other than dehydration. A serum system containing initially 1000 grams of water has been taken as the unit under consideration. By dehydration the water of this serum unit has been reduced to $1000 \times \frac{[Cl]_I}{[Cl]_R}$ where $[Cl]_I$ and $[Cl]_R$ refer to the concentration of chloride in milli-equivalents per 1000 grams of serum water before exercise (initial) and after two hours of recovery. Since the concentrations of electrolytes have been expressed in milli-equivalents per 1000 grams of serum water, the concentrations at exhaustion and recovery have been multiplied by $\frac{[Cl]_I}{[Cl]_R}$ to find the amounts present in the serum unit at exhaustion and recovery. The differences between these values and the initial concentrations have given the changes in electrolytes due to factors other than dehydration.

The application of such calculations to the data of table 1 has given the results shown in table 2. The changes in serum lactate, phosphate and proteinate in these experiments are small and variable, both in amount and direction. During recovery lactate decreases in amount, while phosphate increases. The most marked changes occur in bicarbonate and total fixed base, both of which decrease. The greater changes in bicarbonate occur in experiments in which there is a rise in pH and in lactate. In such experiments a marked rise in bicarbonate occurs on recovery, but seldom to the extent that the initial bicarbonate is restored. In the experiments with dog 5 there is little increase in bicarbonate during the two hour rest period following exercise. Very little change in fixed base occurs during the recovery period in any case. The increase in the anion deficit disappears during recovery.

The data of the experiment with dog 5 shown in figure 1 have been used

to calculate the changes other than those due to dehydration which occur during the course of the exercise period. The results are shown graphically in figure 2. The diagram again shows that the changes in serum lactate, phosphate and proteinate are small; fixed base decreases at first in response to overventilation, decrease in fixed base and increase in lactate; as exercise continues to exhaustion the increase in the anion deficit plays an important rôle. The diagram shows strikingly, however, that for this dog the greater part of the decrease in bicarbonate is a response to the decrease in fixed base.

TABLE 2

*Average changes in the electrolyte pattern of the serum as a result of exercise and recovery**

DOG NUMBER	NUMBER OF EXPERIMENTS	TIME INTERVAL	H_2O OF SERUM UNIT = $\frac{[Cl]_I}{1000 \times [Cl]_R}$	Δ pH	Δ BICARBONATE	Δ LACTATE	Δ PHOSPHATE (× 1.8)	Δ PROTEINATE	Δ CHLORIDE	Δ ANIONS	Δ FIXED BASE	Δ ANION DEFICIT
				From rest to exhaustion								
		minutes	*grams*		*m.-eq.*	*m.-eq.*	*m.-eq.*	*m.-eq.*	*m.-eq.*	*m.-eq.*	*m.-eq.*	*m.-eq.*
3	4	128	939	+0.12	−9.8	+2.0	−1.6	+0.1	+1.4	−7.9	−4.9	+3.0
6	5	133	910	+0.11	−7.5	+0.3	−1.0	0.0	−0.7	−8.9	−6.2	+2.7
5(a)	5	132	941	0.00	−4.4	−0.4	−0.8	−0.9	+0.3	−6.2	−3.3	+2.9
5(b)	11	302	885	−0.02	−6.6	−0.9	+0.4	−1.3	0.0	−8.4	−5.7	+2.7
				From exhaustion to recovery								
3	4	120	939	−0.15	+5.6	−2.7	+1.6	−1.4	−1.4	+1.7	+0.4	−1.3
6	5	120	910	−0.13	+3.5	−1.3	+1.0	−1.6	+0.7	+2.3	−0.2	−2.5
5(a)	5	120	941	−0.03	+1.8	−0.3	+1.4	0.0	−0.3	+2.6	−0.2	−2.8
5(b)	11	120	885	−0.02	+0.9	−0.4	+0.9	0.0	0.0	+1.4	−1.0	−2.4

* Correction has been made for loss of serum water by dehydration. Results are expressed in terms of the initial serum unit containing 1 kilo of serum water.

In a previous report (2) it was shown that the decrease in serum base relative to chloride appears to be due largely to loss of base from the system by way of the kidneys. It was also shown that the amount of bicarbonate excreted by the kidneys increases as a result of exercise, but that the increase in the twenty-four hours including and following the exercise period is only about 1 milli-equivalent. The decrease in serum bicarbonate associated with decrease in fixed base may be interpreted as the result of the elimination of H_2CO_3 by the lungs in response to the excretion by the kidneys of excess base in the form of a dibasic phosphate. The finding (2) that the pH of the urine excreted during the twenty-four

hours including and following exercise tends to decrease slightly rather than to increase does not support, but, at the same time, does not entirely eliminate this possibility, for during the actual exercise period a more alkaline urine may have been excreted. Decrease in serum bicarbonate associated with decrease in fixed base may also be interpreted as the result of the replacement of bicarbonate in the serum by some other anion which in turn is eliminated by the kidneys. Evidence of the increased elimination of inorganic sulphate and phosphate in the urine after exercise supports such a view.

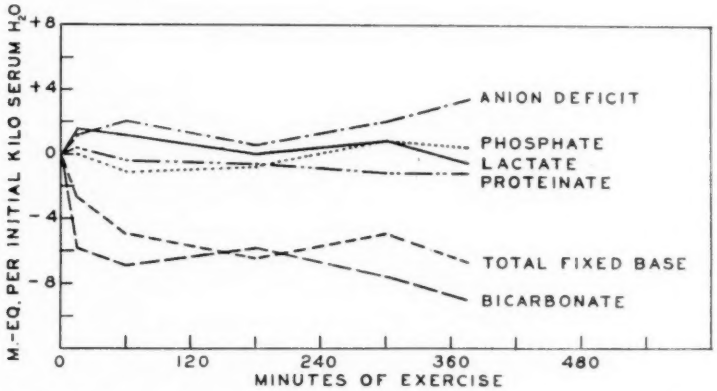

Fig. 2. Changes in the composition of the serum of dog 5 during exercise after correction for loss of serum water by dehydration. Changes are expressed in terms of milli-equivalents relative to an initial kilo of serum water. From data of the experiment in figure 1.

SUMMARY

The changes in the electrolyte pattern of the serum of the dog, resulting from strenuous, long-continued exercise on the inclined treadmill, have been presented.

Effects of dehydration have been taken into account in the calculation of the results by assuming that the increase in serum chloride concentration is due almost entirely to loss of body water and, therefore, that the amount of dehydration of the serum is proportional to the increase in the chloride concentration.

The fall of serum bicarbonate concentration which invariably occurs has been shown to be due only in part to the well known effects of overventilation and of entrance of lactic acid into the blood stream. Changes in the concentrations of phosphate, proteinate, and other anions as yet undetermined produce compensatory changes in the bicarbonate concen-

tration, but a large proportion of the fall in bicarbonate concentration is associated with a loss of base from the fluid system.

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EFFECTS OF AMNIOTIN OR THEELIN ON METABOLISM OF LIVER AND KIDNEY

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In following the metabolism of rat liver, kidney and anterior pituitary through the phases of the oestrous cycle, it has been found that these tissues react in different ways. The metabolic rate of liver and pituitary is higher during oestrus than in dioestrus; that of the kidney remains fixed (8,9). Spaying does not alter the metabolism of the anterior pituitary or kidney from that of the dioestrous state, whereas the metabolism of the liver is lowered.

These observations led to the present study, which deals with the effect of oestrus, experimentally produced in spayed animals by injection of amniotin or theelin, upon the metabolism of liver and kidney.

METHOD. The breeding, diet and care of the rats have been described (1). The animals had free access to food and water. Ovariectomy was performed on the 60th to 62nd day. Six weeks after operation when the animals were about 100 days old, amniotin (Squibb) dissolved in ethylene glycol was injected to a total dose of 30 rat units, administered over 1 or 2 days depending on whether the rats were studied 1 or more days after the first injection. In some cases crystalline theelin, M.P. 225°C. corrected, dissolved in aqueous solution as described elsewhere (10) was injected in 9 gamma doses, 30 r.u., 0.3 γ being equal to 1 r.u. according to Biskind (5). The theelin was kindly supplied by Dr. Oskar Wintersteiner and the amniotin by Dr. A. J. Morrell of the Squibb Research Laboratories. The hormone preparations employed in these experiments produced changes in the vaginal smear 48 hours after the first injection. Oestrous smears were characterized by an abundance of nucleated and cornified epithelial cells. On the third day the smears contained many cornified epithelial cells and on the following day a few white cells. No significant differences between the effects of amniotin or theelin were noted. All animals with infections were excluded by autopsy because it has been found that infection in the host influences the metabolism of liver in vitro (8). Examinations of the middle ears and of the lungs, the most common sites of infection, were made in every case.

At various intervals after injection the rats were sacrificed by a blow on

TABLE 1

The respiratory rates and quotients of liver and kidney at intervals after the intraperitoneal administration of 30 r.u. of amniotin or theelin

DAYS AFTER FIRST INJECTION	LIVER		KIDNEY	
	O_2 consumption	R.Q.	O_2 consumption	R.Q.
	cmm./gm./min.		*cmm./gm./min.*	
0	14.7	0.85	52.6	1.02
	12.8	0.81	52.6	0.80
	11.5	0.75	55.2	0.98
	11.8	0.78	40.5	0.96
	14.4		44.0	0.88
	12.0	0.70	40.9	0.95
	14.8	0.84	48.0	1.00
	14.7	0.76	55.8	0.83
	13.3	0.70	51.7	0.88
1	12.4	0.69	55.0	
	13.3	0.76	57.6	0.90
	13.2	0.80	55.2	0.87
	11.6	0.70	45.4	0.98
	12.1	0.72	43.1	0.74
	10.5		53.7	0.95
	13.6	0.77	63.3	0.88
	13.5	0.77		
2	14.2	0.76	35.9	0.88
	14.2	0.67	43.2	0.97
	19.3	0.80	54.4	1.00
	16.5	0.86	59.6	0.94
	12.0	0.65	39.0	0.88
	11.8	0.74	35.3	0.93
	12.7	0.70	45.6	0.96
	14.9	0.84	51.0	1.00
3	15.9	0.78	53.5	0.97
	10.0	0.81	48.2	1.00
	17.6	0.80	54.8	0.92
	11.8	0.70	56.0	0.98
	15.1	0.80	54.0	0.90
	17.1	0.74	48.2	0.96
	16.3		42.6	0.88
	15.2	0.81	45.3	0.87
	18.6	0.79		
	14.6	0.70	49.5	0.91
	20.5	0.70	59.8	0.82
	17.2	0.85	56.5	0.80

TABLE 1—*Concluded*

DAYS AFTER FIRST INJECTION	LIVER		KIDNEY	
	O_2 consumption	R.Q.	O_2 consumption	R.Q.
	cmm./gm./min.		*cmm./gm./min.*	
4	12.2	0.78	41.4	0.89
	10.4	0.69	45.8	0.87
	11.6	0.76	52.2	0.82
	13.4	0.91	50.0	1.00
	12.4	0.77		
	12.3	0.84	53.8	0.94
	18.1	0.88	57.4	0.88
	13.6	0.77		

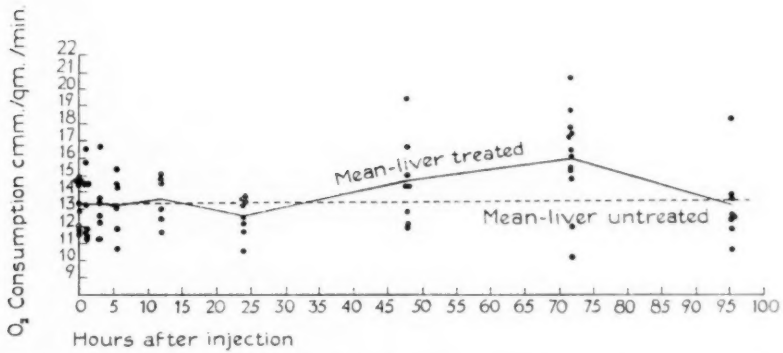

Fig. 1. Scatter chart of individual rates of oxygen consumption of liver at intervals after the intraperitoneal injection of 30 r.u. of amniotin or theelin. Solid line connects the mean rates at various intervals after injection. The dotted line refers to the mean metabolic rate of livers of untreated spayed rats. There is a significant increase on the third day. The second day shows a slight trend toward an increase.

the head. The liver and kidneys were sectioned; the slices weighed and placed in Ringer solution. About 30 minutes elapsed between the death of the animal and the first metabolism reading. The respiratory rates and quotients were measured with a differential volumeter (11).

Results. Only in the liver were there changes in metabolism. The increased respiratory rate was significant on the third and returned to the control level on the fourth day after the hormone injection. However, the scatter chart of the individual observations suggests that changes began to appear two days after injection and may have persisted to the fourth day. There were no significant changes in the qualitative metabolism of either liver or kidney as indicated by the R.Q. The changes in liver metabolism and the unaltered renal metabolism following theelin

or amniotin administration in spayed rats resemble the metabolic state of these tissues at oestrus (8). In these spayed rats injected with the follicular hormones the morphological changes of oestrus in the genitals corresponded with the time of the physiological changes in the liver. Whether the increased liver metabolism at oestrus, in the cycle or in theelin treated spayed rats, is the result of the increased muscular activity of such animals (6,7) cannot be stated from these experiments.

Theelin directly increases the respiration of anterior pituitary but not of liver of spayed rats (10). Furthermore, the "in vivo" reaction of the liver to theelin is contrasted sharply with that of the anterior pituitary.

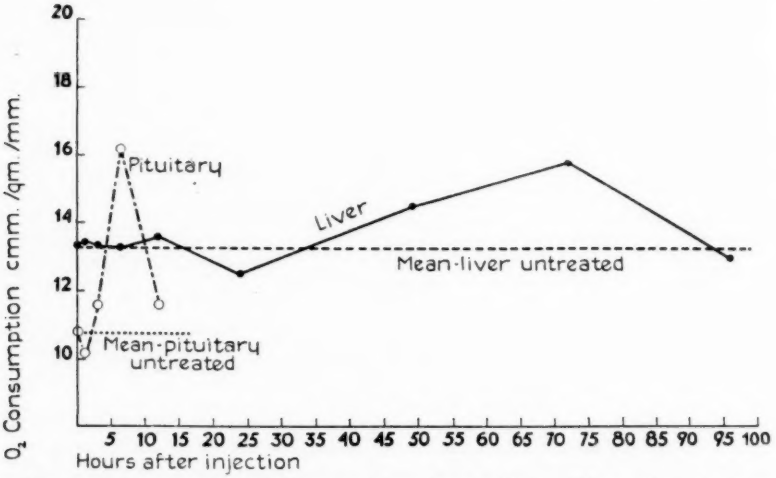

Fig. 2. Comparison between liver and anterior pituitary. Effects of amniotin or theelin administration in spayed rats. Data for first 12 hours from previous reports (10). Pituitary rate increases in 6 and returns to untreated level in 12 hours; increased liver metabolism occurs 72 hours after injection.

There is a striking difference in the time interval for maximum response. Figure 2 shows a comparison of the mean respiratory rates of anterior pituitary and liver following the intraperitoneal injection of 30 r.u. of theelin or amniotin in spayed rats. The data for the first 12 hours of the curves are drawn from a previous report (10). In the rat spayed 6 weeks the anterior pituitary metabolism changes in 6 and returns to the original level in 12 hours; the liver requires 72 hours for its maximum response. The above observations, namely, the diverse "in vitro" and "in vivo" reactions of the liver and anterior pituitary to theelin, indicate that the intermediate stages of the metabolic response in these tissues is quite different.

Discussion. Atrophy of adrenal and thyroid (2) and for a certain time,

the pituitary (3) occurs after ovariectomy. The oestrous picture can be restored by amniotin (4). The maximum weight gain of these glands following amniotin treatment is 1 day for pituitary, 2 days for adrenal and 3 days for thyroid. In the liver the maximum metabolic change coincides with the maximum weight and histological recovery of the thyroid gland. A well known feature of thyroid activity, as judged by thyroid feeding or

TABLE 2

Mean respiratory rates and quotients of tissues at intervals after the intraperitoneal injection of 30 r.u. of amniotin or theelin

DAYS AFTER INJECTION	NUMBER OF RATS	LIVER				KIDNEY			
		O_2 consumption (cmm./gm./min.)		R.Q.		O_2 consumption (cmm./gm./min.)		R.Q.	
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
0	9	13.3 ± 0.40	1.7	0.774 ± 0.012	0.05	49.6 ± 1.33	5.6	0.923 ± 0.016	0.07
1	8	12.5 ± 0.25	1.29	0.744 ± 0.012	0.04	53.3 ± 1.85	6.8	0.887 ± 0.022	0.07
2	8	14.5 ± 0.47	2.0	0.753 ± 0.016	0.07	45.5 ± 1.9	8.1	0.945 ± 0.011	0.05
3	12	15.8 ± 0.14	0.67	0.771 ± 0.01	0.05	51.7 ± 0.31	1.5	0.91 ± 0.013	0.06
4	8	13.0 ± 0.54	2.14	0.80 ± 0.017	0.07	50.1 ± 0.22	1.7	0.90 ± 0.017	0.06

TABLE 3

Differences of mean respiratory rates and quotients of tissues at various intervals after the intraperitoneal injection of 30 r.u. of amniotin or theelin

Significant difference italicized

COMPARISON WITH UNTREATED RATS	LIVER				KIDNEY			
	O_2 consumption (cmm./gm./min.)		R.Q.		O_2 consumption (cmm./gm./min.)		R.Q.	
	Diff.	Diff. / PE Diff.	Diff.	Diff. / PE Diff.	Diff.	Diff. / PE Diff.	Diff.	Diff. / PE Diff.
days								
1	−0.8±0.47	1.7	−0.03 ±0.017	1.8	3.7±2.28	1.6	−0.036±0.027	1.3
2	1.2±0.62	1.9	−0.021±0.02	1.0	−4.1±2.32	1.8	0.022±0.019	1.2
3	*2.5±0.42*	6.0	−0.003±0.016	0.2	2.1±1.37	1.5	−0.013±0.021	0.6
4	−0.3±0.67	0.4	0.026±0.021	1.2	0.5±1.35	0.4	−0.023±0.023	1.0

thyroxin injection, is the lag between the time of thyroid administration and the onset of increased metabolism. In most species this interval is between 24 and 48 hours. If the increased activity of the thyroid were related to the increased metabolism of the liver, certain other relationships would follow. Depending upon the lag period of the rat liver to thyroid secretion, stimulation of thyroid secretion must have occurred 1 or 2 days after amniotin treatment.

The maximum morphological (4) and physiological (10) response following theelin administration occurs at least 2 days earlier in the anterior pituitary than in the thyroid. This relationship suggests that the anterior pituitary may stimulate the thyroid through secretion of thyrotropic hormone. Thyroid secretion, in turn, may then increase the metabolism of the liver.

SUMMARY AND CONCLUSIONS

1. The respiratory rates and quotients of excised liver and renal cortex of spayed rats of the same age and strain were measured before and at daily intervals after the injection of 30 r.u. of amniotin or theelin.

2. The respiratory rate of the liver was significantly increased 3 days after treatment and on the fourth day it returned to the original level. The metabolism of the kidney was unaltered. This effect of theelin on the liver is quite different from that found in the anterior pituitary (10). It occurs "in vivo" but not "in vitro" and becomes manifest after a much longer interval. The mechanism responsible for the increased liver metabolism following amniotin or theelin injection appears to be different from that of the anterior pituitary.

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CHOLINE AS A STIMULANT OF GASTRIC SECRETION

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This work was carried out as a contribution to the study of the second, or "chemical," phase of gastric secretion. The identity of the secretagogue bodies of the food responsible for the second phase is still unknown. The non-specific hydrolytic products of the three main classes of foodstuffs can play no important part: thus the amino acids stimulate gastric secretion either weakly, or not at all (1, 2); the fatty acids excite only after producing a marked inhibitory action; while the sugars are inactive. The extractive substances of foods, especially foods of animal origin, do, however, include bodies of high secretagogue potency, and Krimberg and Komarov (3) have pointed out that among these the nitrogenous bases are probably of especial importance. Campbell (see Babkin, 4), whose results were confirmed by MacIntosh (unpublished), found that practically the entire activity of fish-muscle extracts could be recovered in the fraction of the nitrogenous bases. Further fractionation by the silver-baryta method showed that the activity was due to the "arginine" and "lysine" fractions, the "purine" and "histidine" fractions being ineffective. Of the known compounds present, nearly all can be eliminated: thus histamine does not act from the digestive tract except in massive doses; adenosine is destroyed during tissue autolysis and by digestion; while creatine, carnosine, carnitine, and methylguanidine are too feebly active to be of any importance. Choline, on the other hand, is distributed widely in foodstuffs, and possesses well-marked physiological activity. It has been shown by Ivy and Javois (5) to excite gastric secretion on being introduced into the digestive tract, but no detailed analysis of its action has been made. We have therefore attempted such an analysis, with the aim of determining whether or not the secretagogue activity of choline is sufficient to account, on the basis of the choline content of various foods, for any part of the observed secretory responses of the stomach to these foods.

The effect of choline on the gastric glands is of further importance, since it is desirable to know whether the second phase involves the active participation of the peptic cells. This question has been discussed by Babkin (4). These elements are under the control of the vagus, and choline, since it exhibits parasympathomimetic activity, might be expected to stimulate them.

METHODS. Most of the experiments were carried out on three dogs equipped with pouches of the stomach, as follows:

"P": female, weight 14 kgm., equipped with a Pavlov pouch (vagal and sympathetic innervation intact) and a gastric fistula.

"H": female, weight 21 kgm., equipped with a Heidenhain pouch (sympathetic innervation only).

"B": male, weight 19 kgm., equipped with a Bickel pouch (extrinsic denervation as complete as possible without severing of blood vessels) and a gastric fistula.

The two last-named were used in most of the experiments, since their responses were not complicated by psychical factors, even when food was taken by mouth. When dog "P" was used, test-meals were injected directly into the stomach by gastric fistula; this procedure was also used with "B" when the test-meal was meat. The animals were all kept in good health over a long period. They received a uniform diet of lean beef heart, oatmeal, milk, and salt. All experiments were begun in the morning while the gastric glands were at rest, water having been previously given *ad lib.* Experiments were never begun if the resting secretion exceeded 0.5 cc. for 30 minutes: the daily secretion of the pouch when no food was given did not exceed 3 to 4 cc., practically all mucus.

The test-meals used were minced lean beef in quantities of 300, 500, or 700 grams, unsalted butter (100 grams) with 100 cc. water, and white bread (250 grams) with 200 cc. water. For comparable experiments the same lot of minced meat was used, since different lots vary rather widely in secretagogue potency: control experiments showed that the secretory effect of such meat was not changed by a week's storage in the ice-box. Choline was given as choline chloride (Merck). The lecithin used was a purified preparation supplied by the Department of Biochemistry, McGill University. On the day before each experiment, the animal received the standard meat meal. Experiments were not performed on successive days.

The pouch secretion was collected in the usual way and measured at half-hourly or hourly intervals. The volume of visible mucus in the juice was estimated separately. Free acidity and total acidity were determined by titration with Töpfer's reagent and phenolphthalein respectively. Pepsin was determined by Nirenstein and Schiff's (6) modification of Mett's method, the digestibility of the coagulated egg-white in the Mett's tubes being checked by means of a standard synthetic gastric juice freshly prepared from a commercial pepsin.

Acute experiments, designed to test more exactly the action of choline on the peptic cells, were carried out on dogs anesthetized with a chloralose-urethane (1:10) mixture. Both vagi were cut in the neck, the pylorus was tied, a small silver fistula was sewn into the ventral wall of the stomach, and the stomach was washed out with warm tap-water. Hista-

mine dihydrochloride (0.1–0.3 mgm. per kgm.) was injected subcutaneously at half-hourly or hourly intervals, and gastric juice for analysis collected during 15-minute periods. When a copious secretion had been established, choline chloride (5–20 mgm. per kgm.) was injected intravenously and the collection of juice continued. Acidity and pepsin were determined as above.

RESULTS. *Effect of choline injected intravenously.* Choline chloride (5 to 15 mgm. per kgm.) injected intravenously during the rest of the gastric glands, evoked in all three animals a scanty flow of gastric juice. The juice was of low acidity but rich in pepsin, and contained much mucus. The injection was followed by salivation, lachrymation, and nasal secretion; the pulse was at first slowed and then became rapid and shallow, and there was marked hyperpnea. These effects passed off within a few minutes. Gastric secretion began in about 5 minutes and continued for an hour and a half. The magnitude of the secretory effect may be illustrated by comparing it with that of the animal's regular meal on the preceding day (see table 1); it was of the same order in all three animals (8 experiments in all). Control injections of saline produced no secretion.

Since the secretion produced by choline consists largely of mucus, which does not drain readily from the pouch, and furthermore since the gastric glands at rest hold in their lumina a considerable quantity of pepsin, which may be washed out in the ferment-free fluid coming from the parietal cells, it was endeavored to obtain a clearer picture of the effect of choline on the peptic cells by superimposing its action on that of histamine, which stimulates the secretion of water and HCl but not the secretion of pepsin. Anesthetized dogs were given histamine subcutaneously (0.2 or 0.3 mgm. per kgm. of the dihydrochloride at half-hourly intervals) and choline chloride (5 to 20 mgm. per kgm.) was injected intravenously when the rate of secretion had become approximately constant. The effect of choline was uniformly (5 experiments) to raise greatly the peptic power of the juice produced by histamine, sometimes to a level approaching that of sham-feeding juice. The rate of secretion, which is very high with such doses of histamine, was generally reduced; the acidity also fell off somewhat. These results are illustrated in figure 3, which compares the effect of choline injected into *a*, the portal vein, and *b*, the jugular vein. The corresponding experiments will be discussed in detail below.

Effect of choline introduced into the digestive tract. Of more significance is the effect on gastric secretion of choline introduced into the digestive tract. This effect was conveniently studied by injecting the choline directly into the stomach through the gastric fistula. Given in this way choline, even in very large doses (0.5–1.0 gram of choline chloride in 50 cc. of water) caused no visible systemic disturbances, as it did when given intravenously, but it did again evoke a rather scanty gastric secretion, which as

before was of very low acidity but rich in pepsin and mucus (table 1; see also fig. 2). The secretion began only after a latent period of about 1 hour, and lasted for about 2 hours: this supports the finding of Ivy and Javois (5) that choline acts from the intestine and not from the stomach. Equimolar solutions of NaCl (1 gram choline chloride equivalent to 0.42 gram NaCl) produced practically no secretion (fig. 2). The volume of fluid secreted, exclusive of mucus, never (11 experiments) exceeded 20 per cent of the volume secreted in response to the standard daily meal, and was usually considerably less than this.

Choline on intravenous injection, or on being introduced into the digestive tract, is therefore a weak stimulus for the secretion of fluid and HCl by the stomach, but a relatively strong stimulus for the secretion of pepsin.

TABLE 1

Gastric secretion in dogs "B" and "H" on choline and lecithin

DOG	STIMULATION	VOLUME	MUCUS	ACIDITY		PEPSIN CONCENTRATION	DURATION
				Free	Total		
		cc.	*cc.*	*m. eq./l.*	*m. eq./l.*		*hours*
B	Choline chloride (300 mgm.) intravenously	2.2	1.5	15	51	550	1¾
	Choline chloride (1 gm.) by gastric fistula	5.3	3.6	0	20	350	2
	Lecithin (6 gm.) by gastric fistula	4.8	3.2	0	15	230	2½
	Daily mixed meal (average)	21.0	1.0	118	132	41	6
H	Choline chloride (300 mgm.) intravenously	3.6	1.5	0	39	460	1¼
	Lecithin (6 gm.) by mouth	4.8	2.8	12	30	260	2
	Daily mixed meal (average)	31.0	2.5	106	116	64	6

Effect of lecithin introduced into the digestive tract. The dose of lecithin used in all experiments was 6 grams, which contains theoretically about the same amount of choline as 1 gram of choline chloride. The choline content of the preparation was not checked. The lecithin was emulsified in 100 cc. of water at 37° and given by gastric fistula to dogs "B" and "P", or by mouth to dog "H". The latent period of the secretory effect was about 2 hours, nothing but a little mucus being discharged from the pouch in the meantime. A scanty flow of gastric juice, carrying with it considerable mucus, then began, and continued for 2 or 3 hours. As with the choline secretion, the values for free and total acid were extremely low and the values for pepsin high. The figures obtained for pepsin doubtless do not represent the entire activity of the peptic cells, since *a*, pepsin may have been destroyed by contact with alkaline mucus, and *b*, the volume of clear

fluid secreted was insufficient to wash out all the pepsin discharged into the crypts of the glands; thus, when the animal was subsequently fed or injected with histamine, the first portions of the juice secreted under the new stimulus were unusually rich in pepsin.

The effect of lecithin on the gastric glands is thus qualitatively similar to that of choline. The latent period for the lecithin secretion is long, no doubt since choline is only slowly released from it by the enzymes of the intestine.

Effect of choline and of lecithin on the secretory response to a test-meal. It was noticed early in this work that, after experiments involving the administration of choline, the volume of gastric juice secreted in response to the daily mixed meal was greater than normal. This observation was extended in controlled experiments, and it was found that the secretory response to a test-meal of either lean meat or butter was augmented by previous administration of choline. The effect was not due to an overlapping of the secretion produced by the meal with the secretion produced by the "direct" effect of choline.

In the first series of experiments, dog "B" was placed on a diet consisting of 700 grams of minced lean beef muscle, given in one meal at 12:30 p.m. daily. The same lot of meat was used throughout the experimental series, which lasted 6 days. On the third day of the series, the animal received 3 hours before the meal 1 gram of choline chloride in 50 cc. of water by gastric fistula. On the fifth day it received an equivalent quantity of NaCl (0.42 gram) in the same way (fig. 1). The choline treatment augmented the secretory response to the meat meal by over 50 per cent, the augmenting effect apparently persisting to the next day. The salt control had no such effect. Two similar 6-day series of experiments gave parallel results. The pepsin concentration of the juice secreted on meat, following administration of choline, was high in the first hour only, suggesting that pepsin secreted during the direct secretory effect of choline was being washed out from the glands; thereafter the pepsin values were the low ones characterizing the juice of the Bickel pouch. Figure 2 shows the volume of secretion and the concentration of pepsin in the juice, when the stimulus was *a*, NaCl followed by meat, and *b*, choline followed by meat. The graph appears to indicate that feeding was carried out before the response to choline had ceased; however, nothing but faintly acid mucus was discharged from the pouch during the hour preceding the meal.

For subsequent experiments the animals were kept on the standard mixed diet, and experiments were not performed on successive days. The experiments were carried out in pairs, one control experiment in which the test-meal was given alone, or preceded by water or salt solution, and one experiment in which choline or lecithin was administered before the test-meal. (The administration of water or of NaCl solution did not increase

the secretory effect of the test-meal.) Test-meals of meat or bread were given by mouth; test-meals of butter, and solutions of choline, lecithin or NaCl were always given by gastric fistula. In order to shorten the ex-

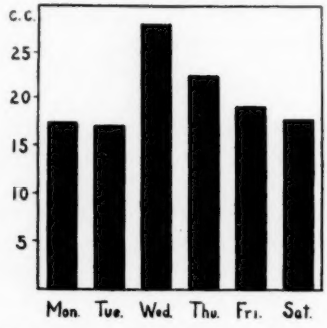

Fig. 1. Dog "B": Bickel pouch. The black rectangles represent the volume of gastric juice secreted in response to the standard daily meal of lean meat. Before the Wednesday meal the animal received 1 gram of choline chloride by gastric fistula. Before the Friday meal, it received an equivalent quantity of NaCl in the same way. Note the persistence of the augmenting effect of choline.

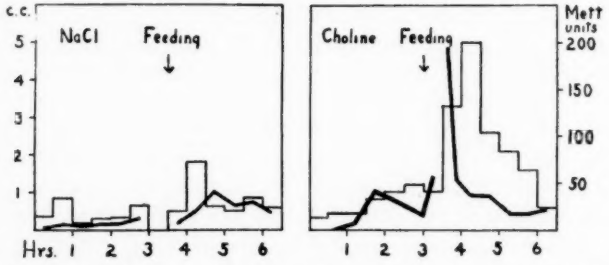

Fig. 2. Dog "B": Bickel pouch. The diagrams show the volume of juice secreted by the pouch (rectangular outlines) and the concentration of pepsin in the juice (heavy lines). The two experiments illustrated were performed two days apart under comparable conditions. In both experiments the test-meal was 700 grams of lean beef-heart, which was preceded in the first experiment by 0.42 gram of NaCl, and in the second experiment by an equivalent quantity (1 gram) of choline chloride. The secretory response to the meat meal was greatly increased by the previous administration of choline. The graph appears to indicate that feeding was carried out before the response to choline had ceased: however, nothing but faintly acid mucus was secreted during the hour preceding the meal.

periments, since the animals became very restless after the end of their normal working day, the test-meal was sometimes administered before the secretion produced by choline or lecithin had ended; since the volume of

clear juice produced by either of these substances did not, in control experiments, exceed 3 cc., this procedure introduced only a small error.

Table 2 summarizes the results of the experiments. Comparable experiments are grouped in pairs. In each set of experiments, it will be seen that the doses of choline (1 gram of choline chloride) and of lecithin (6 grams) which were used, were effective in producing a considerable increase in the volume of gastric juice secreted in response to a test-meal of meat or butter. This is still apparent when the volume of mucus is deducted from the volume of the total secretion. In the two sets of experiments in which bread was used as the test-meal this effect was practically absent. During the first two sets of experiments the dog ("B") was kept on a diet containing no added salt; hence the response to the test-meal (meat) was less than in later experiments. In one experiment (not tabulated) the secretion on meat in the same animal was lessened after lecithin: the animal appeared to be unwell at this time.

The augmentation in the secretion following the test-meal, when choline or lecithin had been administered previously, was due chiefly to an increased flow of juice in the first two or three hours after giving of the meal. The total duration of the secretory response was not affected.

The total output of HCl in all experiments closely paralleled the volume, and hence was higher when choline or lecithin had been given before the meal. The total output of pepsin (concentration x volume) was increased after choline or lecithin, but the increase, since it occurred chiefly in the first part of the test-meal secretion, was probably due, as mentioned above, to a washing-out from the glands of pepsin discharged during the primary effect of choline.

The percentage augmentation of the test-meal secretion by previous administration of choline or lecithin is given in table 3, which summarizes the results of the experiments presented in table 2. In calculating the augmentation, it was assumed that in experiments in which the test-meal was given before the secretion produced by choline or lecithin had ceased, the volume of secretion due to this was 3.0 cc. (the maximum observed in control experiments with these substances alone); and this volume was deducted from the test-meal secretion. The calculation refers to the volume of clear fluid secreted, the volume of visible mucus having been deducted. A similar augmentation by lecithin of the secretion produced by meat given by mouth was observed in the Pavlov-pouch dog (3 experiments).

The effect of choline introduced into the portal circulation. It appeared possible that the weaker secretagogue action of choline on introduction into the stomach, as compared with intravenous injection, might be due to its removal from the circulation by the liver. That the liver does exert such an effect is indicated by two experiments performed on anesthe-

TABLE 2

Gastric secretion in dogs "B," "H" and "P" on meat, butter or bread with or without previous administration of choline or lecithin

DOG	PRELIMINARY TREATMENT	TIME BEFORE TEST-MEAL	TEST-MEAL	DURATION	VOLUME		PEPSIN OUTPUT
					Total	Mucus	
		hours		*hours*	*cc.*		
B	NaCl (0.42 gm.)	3	Meat (700 gm.)	4½	4.8		160
	Choline chloride (1 gm.)	3	Meat (700 gm.)	4½	11.3	(0.6)	805
B	Choline chloride (1 gm.)	3	Meat (700 gm.)	4	13.5	(0.1)	460
	NaCl (0.42 gm.)	3	Meat (700 gm.)	4	9.4	(0.8)	230
	Choline chloride (1 gm.)	3	Meat (700 gm.)	4	15.2	(0.1)	650
B	Choline chloride (0.3 gm.)*	3½	Meat (300 gm.)	5½	22.2	(2.2)	1,180
	NaCl (0.13 gm.)*	3½	Meat (300 gm.)	5½	15.5	(1.7)	750
B	Choline chloride (1 gm.)	½	Butter (100 gm.)	10	19.0	(6.4)	1,595
	NaCl (0.42 gm.)	½	Butter (100 gm.)	10	9.1	(7.7)	925
B	None		Butter (100 gm.)	11	13.9	(7.9)	985
	Choline chloride (1 gm.)	½	Butter (100 gm.)	11	20.4	(7.2)	1,570
P	Choline chloride (1 gm.)	2	Butter (100 gm.)	8	16.0	(3.3)	2,800
	None		Butter (100 gm.)	8	9.6	(1.8)	2,185
P	Choline chloride (1 gm.)	½	Butter (100 gm.)	10½	21.8	(3.2)	4,360
	None		Butter (100 gm.)	10½	8.8	(1.5)	1,280
B	Lecithin (6 gm.)	3	Meat (500 gm.)	6	24.0	(2.5)	800
	Water	3	Meat (500 gm.)	6	17.3	(1.5)	300
B	Lecithin (6 gm.)	3½	Meat (500 gm.)	7	21.7	(2.6)	720
	Water	3½	Meat (500 gm.)	7	11.3	(2.4)	270
B	Lecithin (6 gm.)	½	Meat (700 gm.)	9½	39.8	(2.7)	1,900
	None		Meat (700 gm.)	9½	29.4	(2.9)	1,100
B	Lecithin (6 gm.)	2¾	Meat (500 gm.)	6	22.3	(1.8)	475
	None		Meat (500 gm.)	6	15.7	(1.1)	355
H	Lecithin (6 gm.) + glucose (20 gm.)	4	Meat (300 gm.)	6½	30.8	(5.3)	5,030
	Glucose (20 gm.)	4	Meat (300 gm.)	6	23.4	(3.4)	3,885
H	Lecithin (6 gm.)	½	Meat (700 gm.)	9½	41.4	(2.5)	2,920
	Water	½	Meat (700 gm.)	9½	24.8	(1.3)	1,035
H	None		Meat (500 gm.)	7	22.0	(1.5)	1,145
	Lecithin (6 gm.)	3½	Meat (500 gm.)	7	28.1	(1.5)	2,845

* Injected intravenously.

TABLE 2—*Concluded*

DOG	PRELIMINARY TREATMENT	TIME BEFORE TEST-MEAL	TEST-MEAL	DURATION	VOLUME		PEPSIN OUTPUT
					Total	Mucus	
		hours		*hours*	*cc.*		
B	Lecithin (6 gm.)	½	Butter (100 gm.)	9½	15.9	(8.1)	1,605
	NaCl (0.42 gm.)	½	Butter (100 gm.)	9½	13.9	(8.8)	750
B	Lecithin (6 gm.)	½	Butter (100 gm.)	8½	12.2	(3.6)	660
	None		Butter (100 gm.)	7½	6.9	(5.0)	405
H	Lecithin (6 gm.)	½	Butter (100 gm.)	8	11.1	(3.5)	1,040
	None	½	Butter (100 gm.)	8	4.4	(3.5)	245
P	None	½	Butter (100 gm.)	10	8.8	(1.5)	1,280
	Lecithin (6 gm.)	½	Butter (100 gm.)	10	18.8	(2.9)	3,535
B	None	½	Bread (250 gm.)	10	10.4	(3.9)	795
	Lecithin (6 gm.)	½	Bread (250 gm.)	10	13.8	(6.0)	920
H	None	½	Bread (250 gm.)	8	16.3	(6.5)	2,025
	Lecithin (6 gm.)	½	Bread (250 gm.)	8	22.0	(8.2)	3,795

TABLE 3

NUMBER OF PAIRS OF EXPERIMENTS	TEST-MEAL	PRELIMINARY TREATMENT	AUGMENTATION	
			Range	Average
			per cent	*per cent*
8	Meat	Choline chloride (1 gm.)	44–108	84
7	Meat	Lecithin (6 gm.)	25–116	44
4	Butter	Choline chloride (1 gm.)	50–320	130
4	Butter	Lecithin (6 gm.)	0–400	140
2	Bread	Lecithin (6 gm.)	−10– 10	0

tized dogs secreting under the influence of histamine. In these experiments, in a dog secreting in response to histamine, the same dose of choline chloride was injected slowly into *a*, a branch of the superior mesenteric vein, and *b*, the jugular vein, the technique for collection of juice being as described under "Methods". The increase in the output of pepsin following injection *a* was less than half of that produced by injection *b*, and did not last so long. Reversing the order of the injections did not change the results. Figure 3 represents the results of one of the experiments.

The effect of atropine on the secretory action of choline. The direct secretory action on the gastric glands of choline, given either intravenously or by gastric fistula, is fully antagonized by atropine (0.2 mgm. atropine sulfate per kgm.). Whether or not the secondary augmenting action of

choline is also antagonized could not be ascertained, since atropine interfered with the digestion of the test-meal.

DISCUSSION. The experiments show definitely that besides its direct secretory effect on the gastric glands, choline is capable of augmenting their response to a meal of meat or butter. Lecithin can act similarly. The way in which choline exerts this "augmenting" action on the secretion is not easily understood. It is possible that an increased secretion of pepsin (and possibly of the pancreatic enzymes as well) under the "direct" action of choline, might hasten the escape of secretagogues from the digesting food, and so cause an increased stimulation of the gastric glands. Against this supposition is the fact that the duration of the test-meal secretion is not shortened by choline. It seems more probable that there

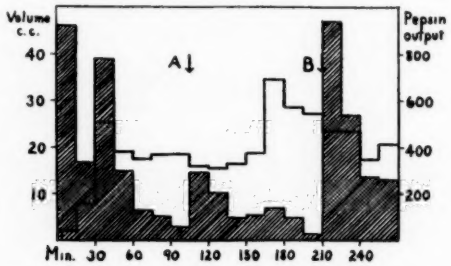

Fig. 3. The heavy rectangular line represents the volume of gastric juice secreted in each 15-minute period by an anesthetized dog receiving 0.2 mgm. of histamine dihydrochloride per kgm. subcutaneously every 30 minutes. The shaded areas represent the total output of pepsin during each period. Note the steady decline in pepsin secretion after the first 45 minutes. At *A*, 100 mgm. of choline chloride were injected into a branch of the superior mesenteric vein; at *B*, the same dose was injected into the jugular vein. Note the greater effect on enzyme discharge of *B* as compared with *A*. Choline had no striking effect on the volume of the secretion produced by histamine.

is some sort of sensitization of the parietal cells themselves, but how this might take place there is nothing to indicate. It is worthy of note that the sensitization affects chiefly the parietal cells, which secrete the fluid and the hydrochloric acid of the juice, and not the peptic cells. The secretory response of the stomach to a standard stimulus may undoubtedly be modified by the state of nutrition of the experimental animal. Thus Koschtoyantz (8) has shown that the prolonged administration of a meat diet increases the response of a denervated pouch to various test-meals; and the addition of liberal quantities of salt to the diet is well known to have a similar effect.

Lecithin is hydrolyzed by the digestive ferments (7), and it is therefore not surprising that its effect should be qualitatively similar to that of

choline. Whether or not choline in the form of lecithin is as effective a stimulus as the free base, cannot be concluded from these experiments, in view of the wide variations between the augmentations observed in the individual experiments. The efficiency of choline and lecithin, when comparable quantities of each are used, appears, however, to be of the same order. The literature does not indicate whether the choline of ingested lecithin reaches the blood as such, or whether it is used to resynthesize the phosphatide in the intestinal wall.

Choline has a stronger secretagogue action when given intravenously than when introduced into the digestive tract. The experiments on intraportal injection of choline suggest that this may be due to its being taken up in the latter case by the liver.

Since the choline or lecithin, as well as any free choline of the diet, can act on the gastric glands, it is of interest to know whether the base is present in the diet in sufficient quantity to be an important factor in the production of the second phase. The doses of choline used in these experiments are certainly much higher than the best figures in the literature for *free* choline in, *e.g.*, skeletal muscle: the true values for the latter are probably very low (9). Recent work has shown, however, that choline is readily liberated by post-mortem autolysis from a water-soluble precursor present in many tissues (9, 10), so that very large quantities of choline have been found in tissues which were not fixed by heating immediately on removal from the body. Thus liver has been found to contain up to 720 mgm. per kgm. (11), and skeletal muscle 150 to 200 mgm. per kgm. (12, 13), even when promptly worked up; this liberated choline is not destroyed by further autolysis. Commercial meats may therefore be presumed to contain very considerable quantities of free choline. The *total* choline of a large number of foods has been determined by Fletcher, Best and Solandt (14). Among other values, liver (dog) was found to contain 2300 mgm. per kgm., beef muscle 750 mgm. per kgm., and white wheat flour 1400 mgm. per kgm. Thus a meat meal of 700 grams, such as was fed in these experiments, contains about 500 mgm. of choline, or about three-fifths the quantity (1 gram of choline chloride) which was found to produce an augmentation averaging 84 per cent in the secretory response to such a meal. The secretion produced by the meat in control experiments was thus probably due, in considerable part, to the "augmenting" effect of the choline which it contained.

In contrast, the "direct" action on the parietal cells of choline derived from food cannot be of much importance in the production of the second phase. Thus in table 1, a dose of choline considerably greater than that which would occur in the daily meal, produced less than one-tenth the volume of (clear) gastric juice produced by the meal itself. On the other hand, the output of pepsin following this dose of choline was twice as

great as that following the meal. Table 2 shows that both lecithin and free choline, in the doses used, markedly stimulate the discharge of pepsin. Since this dose does not greatly exceed the quantity of choline available in a high-protein diet, it is practically certain that the choline of the diet may be a factor in promoting the discharge of pepsin, and that the second phase may involve the activity of the peptic cells. This is evident from the fact that the gastric juice secreted in response to histamine, which stimulates almost exclusively the parietal cells, contains far less pepsin than the gastric juice of the second phase. In the normally innervated stomach, in which the peptic cells undergo intense stimulation through the vagi, this effect of choline would of course be less important.

It is of interest to note that the secretagogue action of choline could be regularly observed in a completely denervated stomach pouch, the test being made at frequent intervals six weeks to fifteen months after denervation. This finding may be contrasted with the statement of Suda (15) that acetylcholine loses its secretory effect on complete denervation of the pouch.

SUMMARY

1. Choline administered to dogs intravenously or by gastric fistula is a weak stimulus for the secretion of fluid and acid by the gastric glands, but a comparatively strong stimulus for the secretion of pepsin.

2. The secretagogue action of lecithin is similar to that of choline, allowance being made for the delay in liberation of choline from lecithin in the gut.

3. The volume of gastric juice secreted in response to test-meals of meat or butter is markedly augmented by previous administration of choline or lecithin. This "augmenting" effect of choline and lecithin is a secondary one, and persists after the direct secretory effect has passed off.

4. The quantities of choline available in the diet indicate that the direct secretory action of choline is not an important factor in the production of the second phase of gastric secretion. The secondary augmenting action of choline, however, plays an important, but subsidiary, part in the production of the second phase.

The authors are much indebted to Dr. B. P. Babkin for suggesting this investigation and for much valuable advice and criticism during its course.

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THE EXCRETION OF INULIN AND CREATININE BY THE ANTHROPOID APES AND OTHER INFRAHUMAN PRIMATES[1]

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In the elasmobranch (dogfish, 11), teleost (red grouper, 8) and bird (chicken, 15) the exogenous creatinine clearance is greater than the simultaneous inulin clearance, and the difference between these two clearances is decreased by raising the plasma level of creatinine or by the administration of phlorizin. These facts have been interpreted as indicating that in these animals creatinine is excreted in part by tubular activity, as in the aglomerular fish (1, 6, 7). But in the dog (10, 12, 14, 19), rabbit (5), seal (17) and sheep (16) the creatinine and inulin clearances are identical, indicating that in these species tubular excretion of creatinine does not occur. Paradoxically, in man only, among the mammals previously examined, is there evidence of the tubular excretion of this substance (13).

In the year 1936, one of us (H. W. S.) enjoyed the welcome opportunity of working in the Laboratory of Physiology of the Yale School of Medicine, and the examination of the excretion of creatinine was begun in some of the animals in the excellent collection of primates which is maintained there. Since it was impossible to complete all the observations desired, the investigations were extended at a subsequent time by R. W. C.

METHODS. None of the animals examined were trained to remain quiet during the manipulative procedures required in the study of renal clearances and it was necessary to make nearly all our observations upon anesthetized animals. Sodium amytal or nembutal was administered intraperitoneally. In a few instances the injection was preceded by light etherization.

In the earlier experiments physiologically inert inulin was administered intravenously in single doses, the plasma concentration at the middle of each urine collection period being interpolated from a semilogarithmic graph. In the later experiments, inulin was given by constant intravenous

[1] These investigations were aided by a grant from the Fluid Research Funds, Yale University School of Medicine.

infusion of a saline solution containing 5 per cent inulin, in order to maintain the plasma concentration of this substance as constant as possible. Creatinine was injected subcutaneously in 10 per cent solution, or included in the intravenous infusion, the plasma concentration being maintained in a fair plateau between 10 and 20 mgm. per cent.

Urine was collected by an in-lying catheter, the bladder being washed out with a measured quantity of water at the conclusion of each period. Blood was usually drawn from a superficial vein but sometimes from the femoral artery. Coagulation was prevented by heparin, and the plasma was separated at once and the proteins were precipitated by the ferric sulphate-barium carbonate method (18). After absorbing the glucose from the filtrates with yeast (4) inulin was hydrolyzed with dilute H_2SO_4 and the resulting fructose determined by the Folin sugar method (3). Creatinine was determined by the Folin-Wu method (2), using a Wratten ϵ 74 filter in the colorimeter. Aliquots from the same filtrates were used for creatinine and inulin analyses. It was demonstrated that neither amytal nor nembutal interferes with the quantitative determination of either substance.

Results. Data on the simultaneous clearances of exogenous creatinine and inulin in the orang-utan, the gibbon, the chimpanzee and the baboon are given in table 1. The creatinine/inulin clearance ratios in these forms, with the possible exception of the baboon, so far exceed 1.0 that it may be concluded that these two substances are handled by the kidney in a different manner, as is the case in man. But only in the orang-utan, the highest of the primates examined, does the creatinine/inulin ratio exceed the average value (1.39) observed in man (13).

The differences between the creatinine and inulin clearances in some periods in the other animals, and particularly in the baboon, are so small that they lie not far outside the possible experimental error. In view of this fact, the biochemical methods were checked in a series of control observations upon a normal dog, by the same experimenter and using the same reagents. In six periods in the dog the creatinine/inulin ratio varied from 0.92 to 1.00 and averaged 0.95. This result would indicate that the ratios observed in the baboon were, in the physiological sense, significantly above 1.0.

In all forms in which the tubular excretion of creatinine has been demonstrated, it has been shown that the difference between the creatinine and the inulin clearances is abolished or considerably reduced by raising the plasma level of creatinine, or by the administration of phlorizin. Before the observed differences in the creatinine and inulin clearances in the primates were accepted as indicating tubular excretion of creatinine, it was felt that these two procedures should be tried upon at least one genus. We hesitated to use the single and very valuable orang-utan, Lulu, for

this purpose, so these observations were made on the chimpanzee (table 2). Here inulin and creatinine were administered by constant intravenous infusion.

In the first part of table 2 there are given observations on the effect of elevating the plasma level of creatinine upon the creatinine/inulin clearance ratio. (The inulin clearance in periods 1 and 2 was low, perhaps because the animal was refractory to amytal and required further light etherization while more amytal was given; the administration of ether was stopped about 60 minutes before the beginning of period 1.) In the control periods the creatinine/inulin ratio (1.25) corresponds with those recorded in table 1 on two other chimpanzees. When the plasma creatinine level

TABLE 1

Simultaneous renal clearances in primates

All observations were made under nembutal anesthesia, 20 to 60 mgm. per kgm.

	SURFACE AREA	NUMBER OF PERIODS	URINE FLOW	CLEARANCE		CLEARANCE RATIO CREATININE / INULIN
				Inulin	Creatinine	
	sq. m.		*cc. per sq. m. per minute*	*cc. per sq. m. per minute*	*cc. per sq. m. per minute*	
Orang-utan "Lulu" (*Pongo pygmaeus*) ♀	1.14	6	3.17–1.16	76.2	112.5	1.48
Lar Gibbon (*Holobates lar*) ♂	0.328	5	3.33–1.13	78.3	95.9	1.23
Chimpanzee "Babe" (*Pan satyrus*) ♀	0.522	6	2.28–0.96	132.6	158.3	1.20
Chimpanzee "Peggy" (*Pan satyrus*) ♀	0.892	6	3.50–1.08	73.3	90.0	1.23
Guinea Baboon #370 (*Papio papio*) ♀	0.522	5	0.44–0.41	71.9	81.6	1.14
Sacred Baboon #369 (*Papio hamadryas*) ♀	0.548	6	1.28–0.43	49.1	57.1	1.17

was raised by introducing creatinine into the infusion fluid this ratio was depressed to 1.0. After three periods at an elevated creatinine level a creatinine-free infusion fluid was substituted; as the plasma creatinine fell to lower levels the creatinine/inulin ratio rose again to values above 1.2. The rise of the creatinine/inulin clearance ratio during the time when the plasma creatinine is falling is at variance with the observations of Shannon upon man, where this ratio remains depressed for a considerable period of time (13). It is not known why the ratio fails to rise as the plasma level falls in man, nor have we any explanation for the rapid recovery of the ratio in the chimpanzee.

In the observations given in the second part of table 2 phlorizin (100

mgm. per kgm.) was administered intravenously at the conclusion of the third period. As in man and the infra-mammals, the drug largely obliterated the difference between the creatinine and inulin clearances. Incidentally, it may be noted that under the influence of phlorizin the

TABLE 2

Effect of elevated plasma creatinine and of phlorizin on creatinine/inulin ratio in chimpanzee, "Lucy" (♀)

Surface area, 1.19 sq. m. Sodium amytal, 90 and 65 mgm. per kgm., respectively.

PERIOD	ELAPSED TIME	URINE FLOW	PLASMA			INULIN CLEARANCE	CLEARANCE RATIO	
			Inulin	Creatinine	Glucose		Creatinine / Inulin	Glucose / Inulin
	minutes	*cc. per minute*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*cc. per sq. m. per minute*		
Effect of elevated plasma creatinine								
From 2 to 8 minutes, 8 grams inulin, 1.2 grams creatinine, 50 cc. water intravenously. 10 minutes to 188 minutes, 5 per cent inulin, 0.5 per cent creatinine, 0.8 per cent NaCl intravenously, 3-5 cc. per minute.								
1	69–90	1.3	152	13.2		72	1.25	
2	90–107	1.5	158	14.1		73	1.23	
5 per cent creatinine added to infusion fluid at 107 minutes								
3	134–150	5.4	178	74.5		99	0.95	
4	150–172	6.0	183	90.0		96	1.00	
5	172–186	6.4	181	101.0		93	1.00	
Creatinine-free infusion fluid returned at 188 minutes								
6	238–256	3.1	165	44.4		97	1.21	
Discarded period								
7	287–305	2.3	164	30.0		92	1.32	
Effect of phlorizin								
From 2 to 7 minutes, 8 grams inulin, 1 gram creatinine, 50 cc. water intravenously. 10 minutes to end as above								
1	27–62	3.2	88	9.4		108	1.22	
2	62–82	4.7	136	11.7		114	1.27	
3	82–100	4.8	153	13.2		106	1.24	
30 grams glucose in 250 cc. water per os, 4 grams phlorizin intravenously at 102 minutes to 117 minutes								
4	132–150	13.3	170	16.3	153	66	1.07	0.93
5	150–168	10.0	175	17.4	138	66	1.06	0.94
6	168–185	8.1	178	18.2	124	68	1.02	0.92

glucose/inulin ratio rose to above 0.90, showing that phlorizin blocks the reabsorption of glucose in the chimpanzee as in all other vertebrates so far examined. The inulin clearance itself was cut nearly in half after phlorizin, a phenomenon which has been observed in most other species,

and which is believed to be due to disturbances in renal circulation. In order to control these results, the same investigator, again using the same reagents, made a similar series of observations on a dog under amytal. In two control periods the creatinine/inulin clearance ratios were 0.99 and 0.97, and in two periods after phlorizin (100 mgm. per kgm. by vein) these ratios were 1.06 and 1.01, and the glucose/inulin ratios were 1.01 and 0.99.

It is concluded from the above observations that tubular excretion of creatinine occurs in the chimpanzee. In view of the large difference between the creatinine and inulin clearances this conclusion may be extended with confidence to the orang-utan, and with a high degree of probability to the gibbon. The evidence in the baboon is admittedly of a borderline nature and, in the absence of confirmatory evidence based upon

TABLE 3

Average inulin and creatinine clearances in the monkey (Macaca mulatta)

Sodium amytal, 27 to 55 mgm. per cent

MONKEY NUMBER	SURFACE AREA	NUMBER OF PERIODS	CLEARANCE		CLEARANCE RATIO CREATININE / INULIN
			Inulin	Creatinine	
	sq. m.		*cc. per sq. m. per minute*	*cc. per sq. m. per minute*	
1 ♀	0.264	3	35.2	57.9	1.07
2 ♂	0.425	5	26.2	25.7	0.98
515 ♀	0.305	6	37.7	45.9	1.20
515 ♀	0.305	6	41.0	44.4	1.09
Observed before anesthesia					
515 ♀	0.305	3	38.2	43.0	1.13
After 50 mgm. per kgm. Na amytal intravenously and intraperitoneally					
		4	24.5	28.0	1.14

the action of elevated plasma creatinine upon the clearance ratios, should be considered as tentative.

Our observations on the macaque monkey are of an inconclusive nature, though not less important for this reason. We have made 27 observations on the macaque, which are given in table 3. The unweighted average creatinine/inulin ratio in these observations is 1.10, with a standard deviation of 0.087, the extremes ranging from 0.93 to 1.28. In the dog the average and the standard deviation of this ratio, obtained by the same or similar analytical methods in the hands of other investigators, has been reported as 0.994 ± 0.034 (12), 0.993 ± 0.048 (14), 1.016 ± 0.0444 (10) and 0.97 ± 0.14 (18). So far as the biochemical methods are concerned it would appear that the creatinine/inulin ratio is significantly higher in the macaque than in the dog; this conclusion is jeopardized, however, by

the obvious possibility that the analytical accuracy and the physiological conduct of the observations may not have been comparable in the several series of investigations on the two species, and that individual macaques may differ in their behavior. In all our observations inulin was administered in a single dose, in consequence of which the plasma level was falling rapidly, and it is possible that an error was introduced into the inulin clearance determinations by this circumstance. Such an error would, however, tend to give a creatinine/inulin ratio below rather than above 1.0. Otherwise we have been unable to find any systematic error in methods which would depress the ratio in the dog or raise it in the monkey.

If tubular excretion of creatinine does occur in the macaque it seemed that it should be discoverable by elevating the plasma creatinine. In a single series of observations of this nature the creatinine/inulin ratio averaged 1.08 (1.07, 1.14, 1.04) in three control periods at plasma levels of 6.45 to 4.55 mgm. per cent; after the elevation of plasma creatinine to a level of 80.0 to 67.0 mgm. per cent, this ratio averaged 1.11 (1.10, 1.13, 1.09). The effect of phlorizin in the macaque was not examined. It was felt that the difference between these clearances, if real, was of such a small order of magnitude that it would scarcely be profitable to pursue the problem at this time.

With regard to the possible effect of the amytal or nembutal upon the tubular excretion of creatinine, there appears to be no reason to suppose that these anesthetics would induce this process in the orang-utan, chimpanzee and gibbon when they had no such effect on the dog, as shown by our control experiment on this animal. On the other hand, there is no reason to suppose that these anesthetics would suppress tubular excretion of creatinine in some macaques while not suppressing it in other macaques, or in the orang-utan and chimpanzee. In a single experiment (table 3, last experiment) we found no difference in the clearance ratio in this animal before and after the administration of amytal (50 mgm. per kgm.). We therefore believe that anesthesia does not complicate our results in this particular respect.

The excretion of endogenous creatinine has been examined in one period in the chimpanzee, Lucy. The plasma concentration was 1.7 mgm. per cent and the excretion rate 2.15 mgm. per minute, giving a clearance of 127 cc. per minute. The calculated creatinine coefficient (30 mgm. of creatinine nitrogen per kgm. per day) is about three times as large as the figure found by Rheinberger (9) on a 20 kgm. immature female, but our animal was not maintained on a rigid creatinine-free diet. Since the clearance of endogenous chromogenic substance is of the same order of magnitude as the clearance of exogenous creatinine, and since in our experiments exogenous creatinine has been administered so that the plasma level is about 10 times the level of the endogenous chromogenic

substance, we believe that no error has been introduced into our creatinine clearances from this source.

Observations on urine flow in the macaque are not recorded, since the bladder was washed out with a quantity of water which in many cases far exceeded the quantity of urine present, and the latter could not be measured accurately. A remarkably large urine flow was observed in the chimpanzee, Lucy, notably in periods 4, 5 and 6 in the second part of table 2. In the other animals the urine flow was relatively small, possibly due to anesthesia.

There are good reasons for believing that the inulin clearances reported here are subnormal, due to the effect of the anesthetic. In our observations on macaque 515 (table 3) the inulin clearance before anesthesia averaged 38.2 (3 periods) and after the administration of amytal, 24.5 cc. per minute (4 periods). In the dog examined by us (R. W. C.) the inulin clearance averaged 69 cc. in 8 periods without anesthesia (two experiments) and in two experiments performed on other days with amytal the clearance averaged 53 cc. per minute (6 periods). From the known effects of anesthesia upon the circulation rate, etc., it is not to be expected that these animals would have clearances of a normal order of magnitude. It is regretted that it was necessary to use an anesthetic in these observations, but the nature of the experimental animals made it imperative, since none of them would tolerate restraint, the larger ones were dangerous, and all of the primates are inclined to evacuate the bladder forcibly under the slightest emotional excitement.

SUMMARY

The simultaneous clearances of exogenous creatinine and inulin have been examined in the orang-utan, gibbon and chimpanzee, two species of baboon and in the macaque monkey. The creatinine/inulin clearance ratio in our limited series of observations averaged 1.48 in the orang-utan, 1.23 in the gibbon and 1.22 in the chimpanzee. It is demonstrated in the chimpanzee that elevation of the plasma level of creatinine depresses the creatinine/inulin clearance ratio towards unity, indicating that the difference between these clearances is due to the tubular excretion of creatinine. Phlorizin abolishes this tubular excretion and blocks the tubular reabsorption of glucose.

In our observations on the baboon and macaque the creatinine clearance appears to be slightly greater than the inulin clearance, but elevation of the plasma creatinine level in one experiment in the macaque did not depress this ratio. If tubular excretion of creatinine occurs in these animals it is of such a small order of magnitude that the creatinine clearance exceeds the inulin clearance by an amount only slightly larger than the experimental error in the determination of these clearances.

We wish to express our gratitude to Prof. John F. Fulton for placing the facilities of the Laboratory of Physiology and its primate colony in the Yale School of Medicine at our disposal, and to various members of his staff and particularly Miss Sarah Chapman and Miss Helen Keigher for assistance in the conduct of these observations.

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STUDIES IN BLOOD VOLUME AND BLOOD PRESSURE FOLLOWING THE EXTRAVASCULAR ADMINISTRATION OF FLUID IN RATS

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In a previous communication Griffith, Jeffers and Lindauer (1) described a transient vascular hypertension following the administration of fluids either subcutaneously or intraperitoneally in rats. This hypertension appeared to be related to increased cerebrospinal fluid pressure. Hemoglobin concentration and hematocrit measurements failed to show evidence of blood dilution correlated with this vascular hypertension. It is this phase of the problem which has been studied further, using plasma protein and blood volume measurements.

METHOD. Procedure A. Fourteen normal albino rats were used, varying in weight from 110 to 240 grams. Glass tubes about 8 cm. long and 1.5 mm. inside diameter were drawn to a tip at one end. Each tube was then coated by the evaporation upon its inner surface of a small amount of a strong solution of potassium oxalate. Under ether anesthesia blood from the tail, about 0.15 cc. in amount, was drawn into such a tube, the end sealed, and the plasma separated by centrifuging. The refractive index of the plasma was then measured, using an Abbé refractometer. Each rat was then given 15 cc. of warm physiologic saline per 100 grams body weight intraperitoneally. After 24 hours, the refractive index of the plasma was again measured in the same way. Plasma dilution was estimated from change in refractive index of plasma using a formula based on the work of Reiss (2) as follows:

$$\text{Per cent dilution} = \frac{(R_1 - R_2)100}{R_1 - R_w - 0.0028}$$

where R_1 is initial refractive index; R_2 is final refractive index; R_w is refractive index of water; 0.0028 is a small correction for the crystalloids of the plasma.

[1] Atwater Kent Fellow in Medicine.

Blood pressure was determined by the method previously described (3).

Procedure B: Twenty-seven normal albino rats were used, varying in weight from 145 to 327 grams. Without anesthesia, each rat was injected with warm physiologic saline intraperitoneally, 15 cc. per 100 grams body weight. After 24 hours blood volume was measured by the vital red method previously described (4) and blood pressure determined. In 6 animals the blood pressure was obtained under ether anesthesia, and in the remaining 21 under nembutal anesthesia.

Results. Figure 1 shows the correlation between percentage dilution based upon refractive index, and blood pressure. Hemoglobin and hematocrit measurements on the same series showed no correlation. The

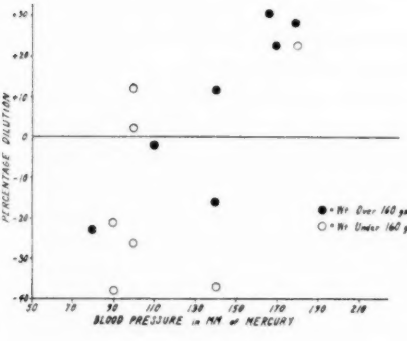

Fig. 1

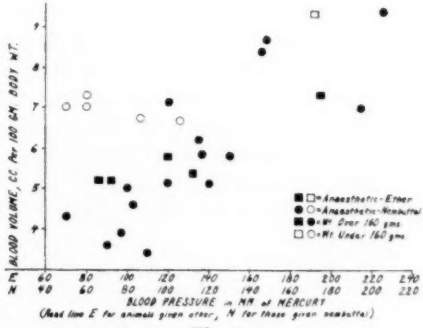

Fig. 2

Fig. 1. Chart showing correlation between percentage dilution of plasma estimated from change in plasma refractive index and blood pressure.

Fig. 2. Chart showing correlation between blood volume and blood pressure (for details see text).

normal range for blood pressure, using ether, is 80 to 140 mm. No animals showed hypertension without evidence of dilution of the blood.

Figure 2 shows the correlation between blood volume and blood pressure. We find the normal range for blood volume by this method to be 4.1 to 5.3 cc. per 100 grams body weight for rats. Animals with a lower blood volume than this were probably, to some degree, in shock. No animals showed hypertension without evidence of increase in blood volume. Because of the use of nembutal in most of the animals two blood pressure scales are used (see fig. 2). The blood pressure with nembutal is approximately 20 mm. lower than with ether, and the normal range is 60 to 120 mm. Hematocrit measurements in this series showed no correlation with blood pressure.

Discussion. A few rats in each group of experiments showed dilution

or increased blood volume but no hypertension. We cannot explain this, but it is suggestive that these rats were small, i.e., young animals. It is possible that a young animal may have a more distensible vascular bed than an older one, and be able to sustain greater increase in blood volume without hypertension.

In our previous paper (1) we attributed the hypertension to increased intracranial pressure. The correlation with blood volume is equally close. It may be that the increased cerebrospinal fluid pressure is dependent upon the increased blood volume.

SUMMARY

Forty-one albino rats were injected intraperitoneally with physiologic saline in amount equal to 15 cc. per 100 grams body weight. After 24 hours 11 animals had an elevated blood pressure, and 30 did not. Of the hypertensive animals, 4 had evidence of blood dilution based upon change of refractive index of plasma, and the remaining 7 had an increased blood volume. Of the animals with normal blood pressure, 18 had no evidence of dilution or of increased blood volume; 8 had evidence of some dilution or increase in blood volume but less than was present in the hypertensive animals; 4 showed evidence of dilution or of increased blood volume to a considerable degree. The 4 in this last group were all young animals. In no case did a vascular hypertension occur without evidence of plasma dilution or of increased blood volume.

Under the conditions of the experiment, blood pressure and blood volume appeared to vary directly.

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THE EFFECTS OF ADRENALECTOMY UPON THE WATER EXCHANGE OF CATS WITH DIABETES INSIPIDUS

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Certain observations of Richter (1), Ingram and Fisher (2) and Keller, Noble and Hamilton (3) indicate that removal or degeneration of the anterior pituitary in animals with experimentally produced diabetes insipidus may greatly reduce the polyuria. It is also known that administration of certain anterior lobe substances produces a marked diuretic effect or may enhance a polyuria already present. It has been suggested, therefore, that the polyuria of diabetes insipidus depends not only upon the absence of functioning posterior lobe tissue but also upon a certain measure of integrity of the anterior lobe. Some workers believe that such action of the pars anterior is exerted through its influences upon other ductless glands. Others postulate the existence of a specific diuretic substance. The effect of various modifications of the endocrine balance upon experimental polyuria is therefore of some interest. Previous communications (Fisher and Ingram, 4; Ingram and Fisher, 5) have described the behavior of the water exchange after thyroidectomy, thyroid feeding, castration, pregnancy and anterior lobe administration in cats with diabetes insipidus. In this paper the results consecutive to adrenalectomy in such cats will be summarized. Justification for such experiments is obvious in view of the known relationship between the adrenal cortex and anterior hypophysis, and of the changes in water distribution and balance resulting from adrenalectomy as shown by Swingle, Pfiffner, Vars and Parkins (6), Harrop, Weinstein, Soffer and Trescher (7), Harrop (8) and others. The latter changes are said to be due in part to loss of water via the kidney.

METHODS. Diabetes insipidus was produced in cats by means of the technique used by Fisher, Ingram and Ranson (9), in which lesions of the hypothalamus produce degeneration of the supraoptico-hypophyseal tract and atrophy of the pars nervosa of the pituitary. The adrenals were removed in two stages by a dorsal approach. Certain of the animals were maintained in good condition for a number of days following the second operation by use of adrenal cortical extract,[1] to insure complete recovery from effects secondary to trauma and anesthesia.

[1] Thanks are due to Dr. David Klein of the Wilson Laboratories, Chicago, Ill., and to Dr. Oliver Kamm of Parke-Davis and Co. for their kindness in providing the active extracts used. We also thank Dr. W. I. Evans of the Department of Anatomy for his assistance in certain of the experiments.

The cats were fed a uniform diet of fresh ground beef heart and milk. Careful daily check was made of the urine output and food and water intake. In summarizing the data the calculated metabolic water and the water content of the meat and milk were included in the fluid intake. No attempt was made to determine the insensible loss of water or the loss of water in feces since these were considered to be fairly uniform in cats under the prevailing conditions.

Observations. Data relating to the water exchange in 2 normal adrenalectomized cats and 2 adrenalectomized controls which had been subjected to brain operations without developing polyuria are summarized in table 1, and expressed graphically for one of these in figure 1. Our control observations confirm the findings of Winter and Hartman (10), also for cats, in that the water exchange diminished along with the onset of adrenal insufficiency, the fall in fluid intake exceeding that of the urine output so that the water balance became negative. The graph (fig. 1), which indicates the daily changes in water exchange, shows this better than does the table, in which the post-adrenalectomy figures are averages based upon the entire survival period without reference to symptoms. Except as portrayed for each day in the graph, an idea of the total negative values developed can only be had by comparing the total post-adrenalectomy water retention with that of the control period corrected for the number of days following the second operation, assuming the retention during the control period to be normal. The total retention figures may be arrived at by subtracting the total output during the period of observation from the intake during that time. Daily retention is the difference between daily intake and output figures. A rough indication of the gross water balance, exclusive of insensible loss, may be had by multiplying the daily retention figure for the control period by the number of days of the post-adrenalectomy period and subtracting from that the total retention during the latter.

The water exchange data before and after removal of the second adrenal in cats with diabetes insipidus are summarized in table 2 and figures 2 and 3. In the case of cat 7 (fig. 2) no extract was used, while cat 8 (fig. 3.) was maintained in good condition by injections of cortical hormone for 8 days. It will be noted that the survival period after adrenalectomy or after discontinuing extract administration was notably shorter than in the case of the control animals. There was a striking and rapid decline in the water exchange, the fall in intake exceeding that in output. Indeed, one receives the impression that the diminished intake was the primary result, for while the urine output declined sharply, it still remained greater than in the control animals until the terminal stages, and in 3 animals it was still well above 100 cc. per day at the time of death. In general, there was a greater discrepancy between intake and output as determined from

daily averages than in adrenalectomized controls. This was not absolute, however, for in one polyuria cat, the average daily retention during the survival period was greater than in any of the controls. Rough calculation of the total loss of fluid during the period after operation or after discontinuing administration of adrenal extract, assuming the retention figures obtained during the control period to be normal, as mentioned above, fail to indicate greater loss than in normal adrenalectomized cats.

Since the survival times of adrenalectomized polyuria cats are shorter than those of controls, and since the loss of fluid is somewhat similar in degree in the two types, one might be led to suggest that this loss via the

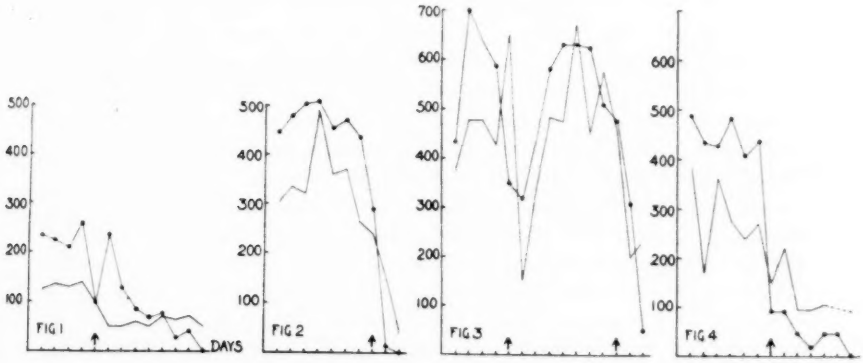

Fig. 1. Water exchange in control cat 2. Arrow indicates day second adrenal was removed. Plain line—urine output.

Fig. 2. Water exchange in cat with diabetes insipidus (7). Arrow—removal of second adrenal. Plain line—urine output.

Fig. 3. Water exchange in cat with diabetes insipidus (8). First arrow—removal of second adrenal. Second arrow—extract discontinued. Plain line—urine output.

Fig. 4. Water exchange in cat 12. Before and during limitation of fluid intake. Arrow—restricted fluid ration begins.

kidney is the cause of death. The shortness of the survival time would then be considered as due to the greater rapidity with which this fatal degree of fluid deprivation was reached. It must be recalled, however, that Winter and Hartman (10) observed similar dislocation of the water balance without fatality in normal, healthy cats the food and water intake of which was gradually reduced by withholding these substances—a finding which led them to state that "the water balance conditions of the total organism have no specific relation to adrenal insufficiency, but are merely a consequence of the nutritional state." Additional light upon this question was therefore sought in studying the water balance of normal diabetes insipidus cats on restricted fluid rations. Data pertinent to such

a study are expressed in table 3 and figure 4. It is quite evident that cats under such conditions do not succumb until they have undergone much greater losses of fluid than occur in adrenal insufficiency. It may be mentioned that ordinary cats are difficult to dehydrate. They may be maintained at least a month without access to free fluid and on a limited diet of thoroughly cooked meat without indications of suffering, and showing only progressive emaciation. They are apparently able to mobilize fluid from the tissues or from tissue breakdown—a capacity probably deficient in adrenalectomized animals. Ordinary diabetes insipidus cats without access to water may die because this mobilization cannot keep pace with the loss via the kidney, but the latter may be greater than that after adrenalectomy without producing fatal dehydration.

Discussion. The results show clearly that bilateral adrenalectomy in cats with diabetes insipidus is followed by decline in the polyuria, although the urine volume does not always fall to as low levels as may appear in normal cats after such treatment. The fluid intake diminishes to a still more marked extent (as is also true of adrenalectomized dogs (6, 7)) and the question arises as to whether or not the fall in urine output is merely secondary to reduced intake. Fisher, Ingram and Ranson (9) have enumerated a number of conditions under which the polyurias of diabetes insipidus cats decline—among these are deprivation of food and water. Failure of appetite is of course characteristic of adrenal insufficiency in cats; this failure occurs early in the polyuria cats and may contribute to the depressed urine volumes. The suppression of thirst is as striking and perhaps as obscure, although it may be caused by the shifts of fluid within the body which have been studied by Swingle and co-workers (11, 12), Harrop (8), Hegnauer and Robinson (13) and others. The rather old idea that a state of thirst depends upon the water content of the body has recently been reiterated by Gilman (14), who found reduction in the water intake of dogs in which cellular hydration was produced by injection of urea. The increase in intracellular water consecutive to adrenal removal (Harrop, 8) would then diminish thirst, reducing the water intake in spite of continued excretion of urine—hence a negative gross water balance.

A further interesting indication that the decline in the polyuria is not altogether a primary thing is found in changes in specific gravity of the urine. If a polyuria characterized by low specific gravity reverts to a normal volume, an elevation of the specific gravity to an approximately normal level would be expected and indeed occurs in cats when Pitressin is administered. We have found that an elevation of such degree does not ordinarily take place in diabetes insipidus cats after adrenalectomy or under conditions of fluid deprivation (see tables 1–3). In only one instance was a normal specific gravity attained—in cat 11, which had a

TABLE 1

Water exchange of control cats

CAT	DAYS	AVE. OUTPUT	AVE. SP GR.	AVE. INTAKE	DAILY RETENTION	TOTAL RETENTION	REMARKS
1	5	102	1.026	151	49	245	Before adrenalectomy
	5	100	1.028	182	82	410	On extract
	8	105	1.027	116	11	88	No extract
	4	60	1.036	45	−15	−60	On extract (inadequate)
	5	40	1.034	36	−4	−20	No extract
2	5	125	1.023	190	65	325	Before adrenalectomy
	8	60	1.034	80	20	160	After adrenalectomy
3							Lesion. No polyuria
	6	112	1.023	139	27	162	Before adrenalectomy
	11	60	1.035	65	5	55	After adrenalectomy
4							Lesion. No polyuria
	9	119	1.025	164	45	405	Before adrenalectomy
	6	44	1.032	63	19	114	After adrenalectomy

TABLE 2

Water exchange of adrenalectomized cats with diabetes insipidus

CAT	DAYS	AVE. OUTPUT	AVE. SP.GR.	AVE. INTAKE	DAILY RETENTION	TOTAL RETENTION	REMARKS
5	8	515	1.004	631	116	928	Before adrenalectomy
	3	163	1.006	127	−36	−108	After adrenalectomy
6	8	300	1.008	364	64	512	Before adrenalectomy
	4	96	1.010	130	34	136	After adrenalectomy
7	8	335	1.007	432	97	776	Before adrenalectomy
	2	94	1.014	8	−86	−172	After adrenalectomy
8	5	482	1.006	526	44	220	Before adrenalectomy
	8	447	1.005	512	65	520	On extract
	2	217	1.009	172	−45	−90	No extract
9	5	423	1.007	491	68	340	Before adrenalectomy
	4	277	1.008	322	45	180	On extract
	3	231	1.007	245	14	42	No extract
10	6	416	1.006	471	55	330	Before adrenalectomy
	4	300	1.009	347	47	188	On extract
	3	192	1.008	186	−6	−18	No extract
11							Mild polyuria
	4	232	1.019	256	24	96	Before adrenalectomy
	6	129	1.024	229	100	600	On extract
	4	82	1.032	89	7	28	No extract

relatively mild polyuria. True, the specific gravities were elevated in the late stages, but the average increases were less than in normal adrenalectomized cats. According to this criterion, then, adrenalectomy does not return the polyuria of diabetes insipidus to normal. The urine volume is diminished, but the decline is in large part dependent upon a fall in intake, and judging by the specific gravity, a polyuria still exists. It seems unlikely, therefore, that an influence of the anterior pituitary in maintaining a polyuria is exerted entirely through the adrenal cortex, and the decline of a polyuria upon anterior lobe degeneration can not be due altogether to a concomitant change in the adrenal gland.

The converse, the effect of diabetes insipidus, or inactivity of the posterior hypophysis, upon the water exchange in adrenal insufficiency is

TABLE 3

Effect of restricted intake on water exchange of cats with diabetes insipidus

CAT	DAYS	AVE. OUTPUT	AVE. SP.GR.	AVE. INTAKE	DAILY RETENTION	TOTAL RETENTION	REMARKS
12	6	283	1.008	425	142	852	Free fluids
	6	126	1.012	51	−75	−450	No free fluid Restricted solid diet Died
6	6	361	1.007	528	167	1002	Free fluids
	6	99	1.016	43	−56	−336	No free fluids Restricted solid diet
	4	335	1.005	435	100	400	Free fluids
8	6	507	1.004	683	176	1056	Free fluids
	4	162	1.012	12	−150	−600	No free fluids Restricted solid diet
	4	541	1.006	751	210	840	Free fluids

worth a brief comment. The chief alteration in the water output and intake curves is the striking rapidity with which they fall and their relatively brief post-adrenalectomy duration. The general relationships of the two curves are essentially similar to those of the controls. The short survival period, of course, attracts attention. The gross physical symptoms of adrenal insufficiency which develop during this time are essentially similar to those characteristic of ordinary cats. Their onset, however, comes sooner after removal of the second adrenal and they develop with considerable rapidity. Symptoms the mildness of which give assurance of no impending crisis in an ordinary animal offer no such favorable prognosis in diabetes insipidus cats. Sudden declines are likely. Forecast survival times are shortened almost from days to hours and even from hours to minutes. A fatal decline may be precipitated by withdrawal of a small

amount of blood or by simple handling. As discussed above, mere total withdrawal of fluid through the kidneys cannot be held accountable for this swift demise, but since the daily loss is increased, the combination of losses in this way with the shift of water into the cells may be a cause for the shortened survival time. Whether this implies more rapid shifts of fluid within the body remains to be determined.

SUMMARY

1. Adrenalectomy in cats with diabetes insipidus results in a rapid decline in fluid exchange. The fluid intake falls more than the urine output and a negative water balance is set up. Extracts of adrenal cortex maintained the water exchange at approximately normal levels when sufficient quantities of extract were given.

2. It appears that the fall in urine output is in large part due to the suppression of fluid intake, as the specific gravity of the urine does not ordinarily reach normal levels even in terminal stages, and the urine volume even on the day of death may exceed normal.

3. The total loss of fluid due to the negative water balance is no greater than in ordinary adrenalectomized cats and is not in itself sufficient to cause death. This is clearly shown by the fact that withholding fluid from otherwise normal diabetes insipidus cats causes greater total loss of water without fatality. Loss of fluid in this way, however, combined with withdrawal of water into the cells as found by other workers in adrenal insufficiency, may be a cause of death.

4. The concurrence of diabetes insipidus with adrenal insufficiency reduces the survival time of the animal.

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THE EFFICIENCY OF THE MAMMARY GLAND IN THE PRODUCTION OF MILK[1]

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The efficiency of the production of milk from nutrients consumed has been a subject of considerable interest for some time. Experiments have shown that if efficiency is measured on the basis of total digestible nutrients, not more than 48 per cent of the food energy may be expected to appear as milk energy. Should the energy for maintenance of the animal be deducted from the total digestible nutrient intake before this calculation is made, approximately 50 to 80 per cent of the energy remaining as unaccounted for may be expected in the milk.

The reactions by which we believe milk solids are formed from blood constituents do not appear to require the amount of energy which appears to be used in the transformation of nutrients to milk substance as indicated in the preceding paragraph. The problems involved in accounting for this energy discrepancy, if it is more than apparent, have been amply discussed in the papers by Brody and his associates (1) to which the reader is referred for a complete presentation of the problems and historical review.

During the course of experiments in which the nutrition of the mammary gland from the circulating blood was under investigation there was occasion to observe the gross respiratory exchange of the gland. The observations were made by analyses of the arterial and mammary bloods for oxygen and carbon dioxide by the methods of Van Slyke and Neill (2), and urea by the method of Van Slyke (3). The technique used has been previously described (4 and 5). Blood samples for oxygen and carbon dioxide determinations were taken under oil and immediately chilled in ice water. Potassium oxalate and sodium fluoride were used as anticoagulants and as a guard against glycolysis. The samples for urea determinations were taken in potassium oxalate alone and similarly chilled.

Table 1 shows the results of analyses of 27 pairs of blood samples taken from five lactating goats, together with the energy value of the milks obtained at the milking which followed the period of blood sampling.

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The results demonstrate that in all but two experiments more carbon dioxide was produced by the reactions taking place within the gland than there was oxygen taken up. Coincident with this finding, the results show that in most cases there was considerably more urea in the mammary venous blood than in the arterial blood.

TABLE 1

Showing analyses of arterial and mammary bloods for O_2, CO_2, and urea with calculated respiratory quotient of mammary gland

EXPERIMENT NUMBER	OXYGEN			CARBON DIOXIDE			R.Q.	MILK ENERGY	ARTERIAL-VENOUS DIFFERENCE, BLOOD UREA	CO_2 FROM UREA, ARTERIAL-VENOUS DIFFERENCE	CORRECTED	
	Arterial	Mammary	Difference	Arterial	Mammary	Difference					CO_2	R.Q.
	mgm. per cent	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*		*cal. per 100 cc.*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	
403	13.004	9.305	+3.699	43.339	48.281	−4.942	1.336	77.000	−0.52	−0.416	−5.358	1.448
348	13.549	7.794	5.755	51.878	56.911	−5.033	0.875	110.462				
30	15.412	12.601	2.811	44.543	48.131	−3.588	1.276	97.817	−1.33	−1.064	−4.652	1.655
403	9.932	5.216	4.716	39.526	46.955	−7.429	1.575	79.104	0.19	0.152	−7.277	1.543
348	11.300	5.130	6.170	46.602	54.400	−7.798	1.264		1.00	0.800	−6.998	1.134
30	11.360	6.388	4.980	49.890	56.000	−6.110	1.227		0.44	0.352	−5.758	1.156
403	13.518	8.170	5.348	45.320	52.090	−6.770	1.266	78.462	0 00	0 000	−6.770	1.266
348	13.411	4.980	8.429	45.470	57.340	−11.870	1.408	91.108	−1.23	−0.984	−12.854	1.525
X	15.200	8.618	6.582	48.200	59.122	−10.922	1.659		−1.57	−1.256	−12.178	1.850
30	10.457	5.885	4.572	52.433	60.134	−7.701	1.684	84.098	−1.02	−0.816	−8.517	1.863
348	9.500	4.668	4.832	49.506	57.350	−7.844	1.623		−1.10	−0.880	−8.724	1.805
403	10.644	6.718	3.926	49.467	55.758	−6.291	1.602	74.097	−1.66	−1.328	−7.597	1.922
30	11.756	7.605	4.151	48.530	53.547	−5.017	1.209	80.397	−6.99	−0.792	−5.809	1.399
403	12.425	6.686	5.739	47.425	54.500	−7.075	1.233	88.863	−0.85	−0.680	−7.755	1.351
348	12.575	6.690	5.885	41.618	51.637	−10.019	1.702		−1.29	−1.032	11.051	1.878
403	11.067	4.851	6.216	53.580	61.933	−8.153	1.312	76.299	−1.17	−0.936	−9.089	1.544
30	11.990	7.927	4.063	52.390	57.536	−5.166	1.271	83.084	−0.07	−0.056	−5.222	1.285
403	14.772	11.049	3.723	44.806	50.750	−5.944	1.597	69.313	−0.78	−0.624	−6.568	1.764
403	15.530	8.685	6.845	50.750	53.805	−3.055	0.446		0.27	0.216	2.839	0.400
30	12.474	7.594	4.880	45.784	50.924	−5.140	1.053	94.245	−0.34	−0.272	−5.412	1.109
30	12.396	7.762	4.634	46.096	52.010	−5.914	1.276		−0.10	−0.080	−5.994	1.293
403	15.201	8.929	6.772	36.629	46.055	−9.926	1.503	51.806	−0.84	−0.672	−10.098	1.610
403	13.804	8.085	5.719	37.812	44.273	−6.461	1.130		0.32	0.256	−6.205	1.085
30	13.341	5.963	7.378	51.640	59.751	−8.111	1.099	68.117	−0.32	−0.256	−8.367	1.134
536	14.847	9.040	5.807	44.332	51.482	−7.150	1.231	66.248	−0.97	−0.776	−7.926	1.365
536	15.095	9.448	5.647	38.375	43.763	−5.388	0.954		0.60	0.480	−4.908	0.869
Aver. ..	12.868	7.530	5.338	46.382	53.241	−6.858		80.654	−0.494	−0.392	−7.250	1.358

The production of urea may complicate the picture of respiratory exchange as determined under the conditions of these experiments since urea may be formed from ammonia and carbon dioxide.

If this type of reaction does take place, the actual production of carbon dioxide would be higher than that as directly determined. Such increases in carbon dioxide production would further increase the value of the re-

spiratory quotient. This correction has been calculated from the experimental results and shown as corrected values for carbon dioxide production and respiratory quotient in table 1.

The classical interpretation of respiratory quotients greater than unity is that the presence of such a condition is the result of fat formation from carbohydrate.

The efficiency of the mammary gland could be estimated from the data in table 1 if the ratio between the amount of blood flowing through the gland and the amount of milk secreted were known. Table 2 shows the results of determinations of the blood volume flow through the mammary glands of five lactating goats. Two of these animals were used in the experiments shown in table 1. Two others were in comparable stages of lactation, while one was in late lactation. These results indicate that the normal ratio of blood flow to milk yield lies between 150 and 250 parts to 1.

TABLE 2

Showing the blood volume flow through the active mammary gland

	TIME	YIELD	BLOOD FLOW	RATIO BLOOD TO MILK
	minutes	*ml.*	*cc. per minute*	
30†	360	325	200.7	223:1
403†	480	315	99.3	151:1
309	340	380	157.0	140:1
309	155	160	158.6	154:1
258*	600	60	40.0	400:1*
X	170	140	145.0	176:1

† Animals used in blood studies.
* Almost dry.

A condition in which the respiratory quotient indicates the formation of fat from carbohydrate does not appear to be compatible with the utilization of substances other than carbohydrate for energy production. Consequently, we may assume the energy of metabolism to be derived from carbohydrate. Lusk (6) has pointed out that the reaction in the formation of fat from carbohydrate is exothermic. The extra carbon dioxide so produced, however, has a caloric value of 1.09 calories per liter as compared with that of 5.01 for the simple oxidation of glucose. Since the CO_2 production and O_2 consumption are identical during the oxidation of carbohydrate, the following formula may be used for calculation of the gross efficiency of the mammary gland.

$$E_F = \frac{EM}{K\ 1.09\ (CO_2 - O_2) + 5.01\ O_2 + EM} \times 100$$

where E_F = gross efficiency in per cent
EM = milk energy per cubic centimeter.
K = ratio of blood flow to milk yield + 100
CO_2 = carbon dioxide in volumes per cent
O_2 = oxygen in volumes per cent

Substituting the mean values from table 1 in this equation and estimating the blood to milk ratio from table 2 as 150 to 1 and 250 to 1, we find a gross efficiency for milk production of 94.9 per cent and 91.9 per cent, respectively.

The technique used in these experiments shows the study of the nutrition of the mammary gland as a separate entity from the remainder of the animal except for losses in any constituent which may be taken up by the lymph.

The results of the experiments reported indicate that the gross efficiency of the mammary gland is slightly more than 90 per cent. The calculations include the maintenance energy of the mammary tissue in addition to the cost of the production of milk itself. Consequently, the cost of transformation of the blood precursors to milk substance is somewhat less than 10 per cent of the total energy transfer.

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LACTOFLAVIN (RIBOFLAVIN) INCREASES HEMOGLOBIN PRODUCTION IN THE ANEMIC DOG

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Various publications dealing with experimental anemia due to blood withdrawal have made it evident that many factors may influence the production of new cells and hemoglobin in these standardized dogs. We may mention iron (13, 7), copper (2) and salt mixtures, globin (12) and amino acids (15), as well as many organ proteins (11). No single key factor has as yet been shown to dominate and control this production of hemoglobin in anemia due to blood loss. The most potent single factor in this type of anemia under all conditions appears to be iron.

In the search for the possible physio-pathological rôle of lactoflavin as one constituent of the vitamin B_2 complex (4), attention has been drawn to the fact that the lactoflavin content of the basal diet used in nutritional control of experimental anemia in dogs is remarkably low. Particular interest has been attributed to the significant finding that fish meat and fish liver, neither of which affects the production of hemoglobin (10), are also practically devoid of lactoflavin. On the other hand, beef and pig liver exhibit the highest anti-anemic potency and similarly the greatest content of lactoflavin.

The parallelism revealed between the anti-anemic values and the lactoflavin content in different foodstuffs is striking and justifies a closer study of the efficacy of pure (natural or synthetic) lactoflavin in the treatment of certain types of anemia.

METHODS. All method details relating to the general anemia program have been described recently (14) and need not be repeated. All animals used in the experiments tabulated below were healthy and clinically normal at the time of these observations. The dogs had been in the anemia colony 6 to 10 years and were consequently very well standardized so that departures from the control hemoglobin production level have real significance.

Crystalline lactoflavin (riboflavin) natural, prepared from whey, or synthetic, was generously put at our disposal by Prof. Richard Kuhn and by

the Winthrop Chemical Company of New York. This material in aqueous solution was given by mouth to dogs in the amounts noted in the tables below. The lactoflavin was added to a small portion of the standard salmon bread ration and fed so that it was promptly and completely consumed. After that the remainder of the diet was placed in the cage. Water was available in the cage at all times.

EXPERIMENTAL OBSERVATIONS. Altogether five experiments have been performed on four different, thoroughly standardized anemic dogs. The results are given in tables 1 to 3 showing all pertinent data. The net output of hemoglobin per two weeks is summarized in table 3. In every experiment it surpassed the range of possible experimental error and should be regarded as a significantly positive response to the lactoflavin administration.

It is apparent that lactoflavin in the doses given (0.1 to 0.5 mgm. per kgm. of body weight daily) has a definite anti-anemic effect which is beyond physiological fluctuations. The changes produced by lactoflavin are of the order of about one-fourth the response to the standard dose of liver (300 grams) which contains about 30 mgm. of lactoflavin. The average response to the lactoflavin is 28 grams of hemoglobin per 2 weeks, which is about one-half the response to the optimum dose of iron (40 mgm. per day)—compare table 3.

Clinical experimental histories—Table 1.

Dog 30-121. Born September 1930. Muscle tissue and salmon bread diet from weaning to adult size. Uneventful routine anemia experiments October 1931 to date.

May 27, 1937—Lactoflavin (synthetic) experiment.

Dog 30-116. Born September 1930. Salmon bread diet from weaning to adult size. Uneventful anemia history September 1931 to September 1936.

August 1936—Lactoflavin (synthetic) experiment. Death October 8, 1936—nephritis.

The hemoglobin production of this dog was normal up to the last month of life, shortly preceding death from nephritis.

Dog 29-67. Born June 1929. Uneventful anemia history September 1930 to April 1937.

September 17, 1934 to May 31, 1935—Plasmapheresis experiment with almost daily exchanges for plasma depletion with only slight clinical reaction. Hemoglobin 85 to 95 per cent during this period. During this period various supplements were added to low protein basal ration (8, 9).

June 19, 1935 to April 6, 1937—Salmon bread diet with routine anemia experiments.

January 28, 1937—Lactoflavin (synthetic) experiment.

The changes here recorded are comparable with those brought about by some amino acids, such as natural and synthetic histidine and phenylalanine (15). In the case of the amino acids, however, this result has been

achieved by administration of daily doses about 100 to 500 times larger than those given in the experiments with lactoflavin. In this connection

TABLE 1

Lactoflavin (synthetic) by mouth increases hemoglobin production

DIET PERIODS 1 WK. EACH	FOOD CONSUMED	WT.	PLASMA VOL.	R.B.C.	R.B.C. HEMAT.	BLOOD HB. LEVEL	HB. REMOVED BLED
Dog 30-121. Coach, male, adult							
Food, gm. per day	%	*kgm.*	*cc.*	*mil.*	%	%	*gm.*
Bread 450, salm. 50, kl. 20	100	15.8	987	4.4	21.9	47	1.4
Lactoflavin (S) 0.5 mgm. per kgm.*	100	15.5	838	5.3	21.4	49	45.6
Lactoflavin (S) 0.5 mgm. per kgm.*	100	15.6	1003	4.1	21.5	48	20.5
Bread 450, salm. 50, kl. 20	100	15.6	904		21.9	52	9.2
Bread 450, salm. 50, kl. 20	100	15.6	915	4.7	19.0	40	31.2
Basal output 14 gm. Hb. per week. Total net Hb. output 48 gm. Total Hb. 106.5							
Dog 30-116. Coach, male, adult							
Bread 425, salm. 50, kl. 20	100	16.5	1016	3.4	18.6	45	1.6
Lactoflavin (S) 0.5 mgm. per kgm.**	96	16.6	988	4.4	19.2	50	28.2
Lactoflavin (S) 0.5 mgm. per kgm.**	100	16.0	915	3.9	17.8	49	39.9
Bread 425, salm. 50, kl. 20	88	15.6	1004	3.0	17.8	49	1.4
Bread 425, salm. 50, kl. 20	100	15.7	1027	3.2	18.8	48	1.3
Basal output 10 gm. Hb. per week. Total net Hb. output 34 gm. Total Hb. 70.8							
Dog 29-67. Coach, male, adult							
Bread 300, salm. 150, kl. 20	80	18.6	927	4.2	19.6	47	1.3
Lactoflavin (S) 0.5 mgm. per kgm.†	100	18.2	922	5.2	22.6	50	25.3
Lactoflavin (S) 0.5 mgm. per kgm.†	100	18.5	971	4.7	20.6	47	22.3
Bread 250, salm. 175, kl. 20	100	18.1	963	4.8	19.0	41	24.2
Bread 250, salm. 175, kl. 20	100	18.0	935	4.2	19.9	45	1.2
Basal output 13 gm. Hb. per week. Total net Hb. output 19 gm. Total Hb. 73.0							

* Bread 450, salmon 50, Klim 20. ** Bread 425, salmon 50, Klim 20. † Bread 250, salmon 175, Klim 20.

Bread = standard salmon bread. Salm. = commercial canned salmon. Kl. = Klim, a commercial skim milk powder.

it should be emphasized that the amount of lactoflavin effective in this type of anemia is the lowest yet demonstrated for a well-defined organic

substance available in synthetic form. Furthermore, these experiments represent a direct contribution to an understanding of the physiological function of lactoflavin. They cast new light also on its nutritional importance as a constituent of the vitamin B_2 complex.

Clinical experimental history—Table 2.

Dog 27-238. Born February 1927. Continuous anemia history November 1928 to August 1937. Diet at no time contained potent animal protein substances in an effort to produce dietary anemia. Experiments consisted of testing vegetables, minerals, drugs and amino acids.

February 1, 1936—Natural lactoflavin feeding.

March 6, 1937—Synthetic lactoflavin feeding.

TABLE 2

Lactoflavin (synthetic and natural) increases hemoglobin production

Dog 27-238. Coach, female, adult

DIET PERIODS 1 WK. EACH	FOOD CONSUMED	WT.	PLASMA VOL.	R.B.C.	R.B.C. HEMAT.	BLOOD HB. LEVEL	HB. REMOVED BLED
Food, gm. per day	%	*kgm.*	*cc.*	*mil.*	%	%	*gm.*
Bread 350, salm. 75, kl. 20	100	16.9	966	4.9	21.2	48	1.4
Lactoflavin (N) 0.1 mgm. per kgm.*	100	16.7	891	5.9	22.0	49	31.1
Lactoflavin (N) 0.1 mgm. per kgm.*	100	16.8	943	5.1	19.5	46	13.1
Bread 350, salm. 75, kl. 20	100	16.9	914	5.0	22.0	47	25.7
Bread 350, salm. 75, kl. 20	100	16.8	974	4.5	22.0	47	1.3
Basal output 12 gm. Hb. per week. Total net Hb. output 22 gm. Total Hb. 71.2							
Bread 300, salm. 125, kl. 20	100	16.0	942	3.5	19.4	45	1.2
Lactoflavin (S) 0.5 mgm. per kgm.**	90	14.4	811	4.6	21.9	55	10.3
Lactoflavin (S) 0.5 mgm. per kgm.**	97	15.1	885	4.6	18.7	45	20.6
Bread 275, salm. 125, kl. 20	93	15.2	962	4.4	17.9	38	23.3
Bread 250, salm. 150, kl. 20	85	14.9	900	4.7	21.4	45	1.3
Basal output 10 gm. Hb. per week. Total net Hb. output 16 gm. Total Hb. 55.5							

* Bread 350, salmon 75, Klim 20. **Bread 275, salmon 125, Klim 20.

Objection may be raised that the basal diet used in the experiments here reported was not completely free from lactoflavin and that consequently in order to evaluate the possible effect of lactoflavin the proportion of the amount of supplemented lactoflavin to the amount of lactoflavin in the experimental diet must be considered. The fact that no such objections were made to experiments in which iron or copper was added to various

diets in anemia due to loss of blood or to deficient diets does not relieve us of the necessity of further investigation of possibly erroneous conclusions. By simple computation, however, it can easily be shown that the intake of lactoflavin in dogs on the basal diet alone is very low. On the basis of biological tests carried out in earlier experiments (6) on different samples of yeast, milk, bran and salmon, the lactoflavin content of the basal diet used in the experiments reported here varied between approximately 0.8 mgm. and 1.5 mgm. daily.

In spite of the positive results shown in tables 1 to 3, the proper function of lactoflavin in formation of hemoglobin or red blood cells remains undisclosed. It is important to note that no sign of anemia could be seen in rats fed a lactoflavin free diet (5). In pernicious anemia, administration of lactoflavin has been of no avail (1, 3). Because of these negative findings it appears that lactoflavin is not a specific catalyst of hemoglobin.

TABLE 3

DOG NO.	LACTOFLAVIN DAILY ORAL			CONTROL NET HEMOGLOBIN OUTPUT PER 2 WEEKS		
	Type	Dose	Hemoglobin net output per 2 wks.	Iron 40 mgm. daily oral	Liver 300 gm. daily oral	Basal bread ration alone
		mgm.	*gm.*	*gm.*	*gm.*	*gm.*
30-116	S	8.0	34		92	20
29-67	S	10.0	19	66	101	26
30-121	S	8.0	48	54	98	28
27-238	S	8.0	16	59		20
27-238	N	1.7	22	63		24

We may choose to believe that the lactoflavin in some way aids in the linkage and aggregation of the amino acids and other materials which make up the large hemoglobin molecule.

SUMMARY

Lactoflavin (natural or synthetic) in daily doses of 1.7 to 10.0 mgm. (0.1 to 0.5 mgm. per kgm. of body weight) causes a definite increase in hemoglobin production above the basal level in standardized anemic dogs. This increase is of the order of one-fourth the hemoglobin production effected by daily feeding of 300 grams of pig liver. These experiments contribute to a better understanding of the physiological function of lactoflavin.

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RECIPROCAL INNERVATION OF THE SPHINCTER AND DILATOR PUPILLAE

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In a study of reciprocal innervation of the iris musculature of the rabbit's eye we wished to measure both the maximal and minimal diameters of the pupil, and to find the rate of dilatation during dark adaptation. This would involve measurements in total darkness which can best be accomplished by infrared photography (Clark, 1934). Red light as a source for photographing the pupil has been used by Lythgoe (1932) and others, who have shown that such light has slight pupillo-motor effect.

The incandescent source of light used in our experiments was the 30-volt, 30-ampere projection lamp, operated from a transformer. This type was chosen rather than a 115-volt lamp because an excellent optical system of high efficiency is available to use with this source (Bausch and Lomb "Cinephor" condensing system). The 900-watt lamp, a spherical, silvered-glass mirror, and two aspheric condensers were mounted in a box which was designed to provide adequate air circulation through a system of staggered baffles and still remain light proof. The radiation exit window of the box was some ten inches from the second condensing lens. In contrast to the usual procedure in infrared work where the filter is on the lens or in the camera, we placed a Wratten no. 87 gelatin filter, mounted dry between two carefully cleaned glass plates at the exit window of the box. The diameter of the effective opening of the filter must be greater than the cross section area of the principal collimated beam so that there will be free egress for the intense heat rays. The gelatin film wrinkles but otherwise remains unharmed as long as it is free from smears or opaque spots which would absorb radiant energy and cause local heating.

The voltage to the lamp was controlled by adjustable rheostats so that it could be operated at a red rather than a white temperature. The energy input to the filament was measured by a wattmeter in the 30-volt circuit. The range of adjustment provided infrared plus considerable visible red through a series to a "pure" infrared with so little of the visible

[1] The method of photographing the pupil was developed by J. E. Gullberg and I. H. Wagman.

spectrum remaining that a human dark adapted eye could no longer detect a white object placed in the direct beam of the lamp. Operation at 600 watts gave a nice balance for photography; there was a high infrared intensity together with a faint far-red component which gave sufficient illumination to permit the checking of the position of the animal. Experiments showed that the residual of visible red left in the light when operating at 600 watts had no appreciable effect on the pupil of the eye, e.g., when the eye was fully dark adapted it was illuminated by the red light for as long as 30 seconds without change in pupil diameter (fig. 1).

The requirements in the camera were rather specialized to meet the severe conditions of accurate focus and framing with a large aperture lens and a sufficient speed of operation to get pictures in a rapid sequence.

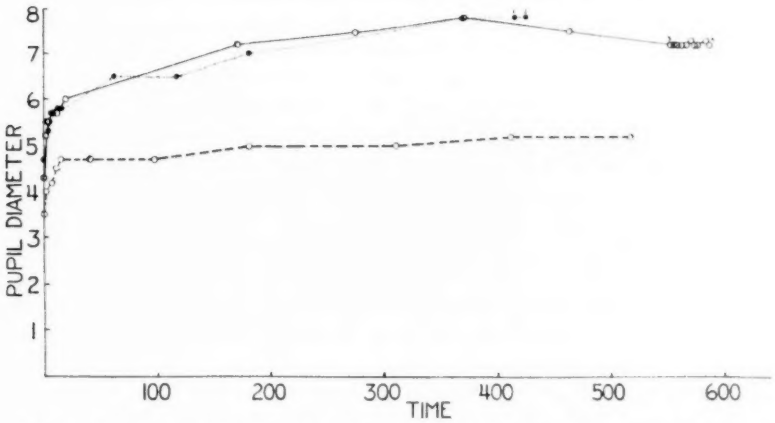

Fig. 1. Curves showing dilatation of pupil of rabbit's eye. Upper curves, normal; lower curve, after cutting the cervical sympathetic. At the end of upper curves, infrared light was left on continuously for at least thirty seconds.

The camera used was a model G Leica fitted with the f:1.5 Xenon lens and a rapid winder attachment. It was necessary to make an offset bracket to provide full clearance for the rapid winder when mounted on a tripod. In its normal mounting the minimum distance which the lens will focus is 3.5 feet. To secure large enough pictures of the eye to permit measurement of pupil diameter it was necessary to mount the lens on the Leitz extension tube "M 1.2". When so mounted the lens at "infinity" focuses objects that are about 30 cm. from the back of the camera. Because the depth of focus of an f:1.5 lens is very limited and the focal point for infrared does not coincide with the visual focus, it was necessary to determine the true object distance. This was done by photographing a test object consisting of a group of cards, each 1 mm. thick, with tabs at the top, and so spaced

and staggered as to present a series of receding indices numbered to correspond to their increasing distance from the camera. When the correct focal distance had been determined, a 10-gauge aluminum wire was mounted in a holder which fits the clip on the top of the camera, and bent so that its tip would define the physical place in space where an object would be both in focus and in the center of the camera field. This method of "physical" focusing is the only one that can be used under conditions of virtual darkness, and with this arrangement it is possible to replace an animal into position in the faint red light. To define the exact time when the pictures were taken, an electrical signal magnet writing on a smoked drum was actuated by a specially devised camera shutter cable release.

It was found that the eyes of albino rabbits did not photograph well; therefore dark-eyed rabbits were used throughout these experiments. The animal was placed in a box in such a position that the light struck the eye obliquely, the rays falling on the peripheral retina. This was an added precaution since the periphery of the eye is least sensitive to whatever pupillo-motor effects the light might have (Lythgoe, 1932). The eye was centered so that its plane was parallel to the plane of the lens of the camera giving a true picture of the eye. A small intense light from a Leitz Mignon lamp was thrown into the eye, and left there until there was no further pupillary constriction. When the pupil was at this minimum, the infrared source was turned on. The next step was to extinguish the white light and simultaneously start the series of photographs.

During the first twenty or thirty seconds, when the rate of dilatation is the most rapid, a picture was taken at intervals varying from two to six seconds. After this first rapid dilatation, a picture was taken, on the average, every minute.

The infrared light was turned off immediately after the first few pictures were taken, and a metal shield was placed in front of the lamp housing. Several seconds before each succeeding picture was to be taken, the light was turned on with the shield still in place. When the filament was emitting its maximum energy, the shield was removed, and the picture taken immediately after. The shield was put back into place, and the light was turned off once again. By this procedure the animal was exposed for only a few seconds to the heat emitted by the lamp, and its comfort in this respect was assured.

The film used was the Agfa infrared, 35-mm. film. An exposure of one-eighth of a second was used with a lens aperture of f:1.5. It was found that an exposure of one-twentieth of a second would give a good image, but it was not as suitable for measuring the diameter of the pupil. The films were developed for seven or eight minutes, at 22°C., in Eastman "D 19" in order to get a negative with considerable contrast. In this way the outline of the pupil was made sharper, making the measurements more exact (fig. 2).

The pupil was measured in the following manner. The test object contained elements whose dimensions were accurately known, and this object was photographed at the end of each series of pupil pictures on the same film strip. The diameters of the pupil were measured directly from the negative with a compound microscope of the "Brinell" measuring type.

Three rabbits, giving results on six eyes, were used for the final experiments. First both eyes of the animals were photographed; then in two animals the left sympathetic was cut, and photographs taken again of both eyes. The right sympathetic was cut a week later in one, two weeks later in the other, and photographs were again taken. In the third rabbit, after the first photographs, both sympathetics were cut in a single operation.

Representative curves are shown in figure 1. A total of sixteen curves from photographs of normal eyes and nine from eyes after cutting the

a *b* *c*

Fig. 2. Enlargements from a typical series of infrared photographs of the eye of a rabbit. *a*, first picture taken from extinguishing the white light; *b*, an intermediate stage in the dilatation; *c*, terminal picture in the series.

sympathetic were plotted and showed the same essential features as these curves. For all normal eyes the curves rise rapidly for about twenty seconds until the pupil has reached more than half its maximal dilatation; the curves then flatten out to a fairly horizontal plateau after three to seven minutes. For eyes of rabbits with the sympathetic cut, the curves showed a similar rise for the first twenty seconds, but the rate is distinctly slower than for the normal eye, and the plateau which follows the rise begins very much more abruptly, sometimes as early as twenty seconds, and is maintained at a much lower level than for normal eyes.

In our opinion there are two forces only which can cause dilatation of the pupil: 1, active contraction of the dilator, and 2, passive stretch of elastic tissues which tend to recoil and restore the resting configuration. Both these forces can come into play in the normal eye, while in the eye with the sympathetic cut, only the elasticity of the tissue can act.

Since the rate of dilatation of the normal eye is always faster than for the eye with the sympathetic cut, this difference must be due to contraction of the dilator muscle aiding the elastic recoil of the tissues. Comparison of the curves shows that in the normal eye the dilator muscle must begin to contract at the very start of dilatation or at least within three seconds.

When the data are plotted as logarithm of time against logarithm of pupil diameter (fig. 3), it was found that (with the single exception of one series), the points for increasing pupil diameter during dark adaptation after the cervical sympathetic was cut, fell fairly well along straight lines. Calculating the slope of these lines by the method of least squares for several series of results on the same eye showed that the slope of these

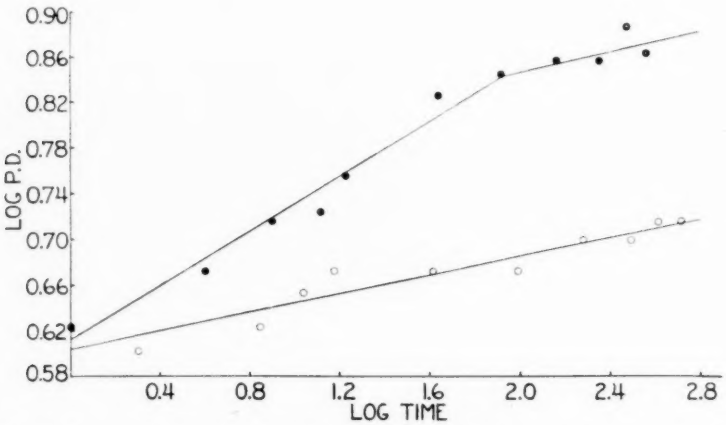

Fig. 3. Log pupil diameter plotted against log time. Upper curve, normal; lower curve, cervical sympathetic cut.

straight lines was the same. This indicates that in all probability the mechanism producing dilatation is the same throughout, and as we have seen, this must be elastic recoil. The steeper slope of the first part of the logarithmic curve for the normal eye as shown both by inspection and by application of the method of least squares (fig. 3), is indicative that, in addition to the elastic recoil which acts alone in the sympathectomized eye, in the normal eye contraction of the dilator fibers assists in dilating the pupil.

The sudden plateau of figure 1 in the case of the eye with the sympathetic cut must represent the limit to which the elastic recoil can dilate the pupil. According to Blier (1928) its pull must now be balanced by a certain degree of residual contraction or tone, which he found to remain

even after the strongest reflex (sciatic) stimulation. He says that "in no case could we produce complete relaxation of the constrictor fibers, regardless of how strong the stimulating current was." In other words, in his experiments, sensory stimulation never gave the degree of dilatation which resulted from stimulation of the head end of the sympathetic or application of atropine. If Blier is correct, then the plateau representing the degree of relaxation of the sphincter in the fully dark-adapted eye after cutting the sympathetic cannot be the limit to which the sphincter is capable of relaxing, and atropine instilled into such an eye should cause further relaxation. This we have tried with the following results. Atropine causes the sympathectomized dark-adapted eye to dilate to the normal maximum. When applied to the normal eye, it does not cause any further dilatation than that in complete dark adaptation.

The only explanation that we can offer for these results is that the smooth muscle of the sphincter must retain a certain degree of tonus even after central inhibition is complete so far as reciprocal action with the dilator is concerned. This suggestion has its parallel in the action of isolated gut where "there is as yet an unexplained relationship between gut tonus and peristaltic movements" (Henderson and Roepke, 1937, p. 379); and again in the postulate of Henderson and Roepke (1934–35) that in the urinary bladder there are "two mechanisms, a contractile one (not depressed by atropine) and a tonus mechanism (readily depressed)."

The question arises whether this residual contraction or tonus is under control of the central nervous system or is a local mechanism. The finding of Poos (1927) that atropine causes a fall in tone of the isolated sphincter pupillae and the fact that in the isolated eye of the eel and frog atropine causes dilatation of the pupil, indicate that it is a local mechanism related to the properties of smooth muscle.

In our experiments this degree of tonus or residual contraction of the sphincter was constant for a given eye completely dark adapted on different occasions, e.g., a diameter of 5.3, 5.3 mm. was found for one eye, 5.3, 5.0, 5.2 for another, etc. If our assumptions which are based on Blier's findings are correct, from this point on the dilator muscle in the normal eye must be acting alone, and this dropping out of the elastic recoil component is signified by the change in slope in the logarithmic curve for the normal eye in figure 3 at the end of about twenty seconds. The latter half of dilatation therefore seems to be carried out by the dilator muscle alone, pulling against the residual tone of the sphincter, unless there is something intrinsically different in the mechanism of the light reflex and the pain reflex, so that in the former advantage can be taken of the possibility of inhibiting the residual contraction. This seems unlikely since the maximum dilatation for complete dark adaptation of eyes with the sympathetic cut is never much more than $\frac{2}{3}$ to $\frac{3}{4}$ of the maximum dilatation

for the normal eye (table 1), although maximum constriction seems to be about the same for both. In dark adaptation, therefore, the action begins by simultaneous contraction of the dilator and relaxation of the sphincter, but when dilatation is about $\frac{2}{3}$ complete, relaxation of the sphincter ceases, but contraction of the dilator continues.

It was found that for the normal eye the part of the curve beyond the rapid rise (fig. 1) was not as smooth as in the rabbits with the sympathetic cut. When the normal eye is adapted to a given intensity of light, it has been found that the pupil diameter undergoes constant slight changes, due, according to Anderson's studies on hippus (1903), to the influence of the cerebral cortex upon centers in the midbrain which alternately excite and relax both sets of muscles. After cutting the sympathetic, during dark adaptation there is central inhibition of the sphincter, but no motor impulses to the dilator, and this evidently results in a more steady state than in the normal eye.

TABLE 1

Summary of the minimum and maximum diameters

(Where more than one series was taken, the figure represents an average)

	NORMAL	LEFT SYMPATHETIC CUT	BOTH LEFT AND RIGHT SYMPATHETICS CUT
Rabbit 1:			
Left eye	4.0-7.4	2.6-5.1	3.3-5.3
Right eye	4.5-7.8	3.3-7.7	3.5-5.2
Rabbit 2:			
Left eye	3.5-7.0	2.7-5.3	2.3-5.3
Right eye	2.6-5.4	3.0-6.2	3.0-4.8
Rabbit 3:			
Left eye	3.8-6.7		3.5-5.7
Right eye	2.8-6.7		3.2-5.3

It was noted that there were individual differences as well as day to day variations in pupil response, but these differences as seen in figure 1 were not serious for purposes of comparison. There was no effect on the opposite eye of cutting one sympathetic and no sign of a consensual light reflex in the rabbit.

SUMMARY

An infrared method of photography is described which enables one to follow the rate of change of pupil diameter during dark adaptation. If certain assumptions are accepted, the dilator muscle should contract throughout the whole course of dilatation, but relaxation of the sphincter ceases after approximately twenty seconds when the process is about two-thirds complete. Dilatation of the pupil is slower and never so complete if the sympathetic nerve is cut. The two eyes are independent, there being no traces of consensual light reflex.

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THE RÔLES OF ANTERIOR PITUITARY AND THYROID IN THE STIMULATION OF TISSUE METABOLISM FOLLOWING THEELIN ADMINISTRATION

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Theelin administration in spayed rats increases the metabolism of the anterior pituitary within 6 hours (27) and of the liver only after 72 hours (3). The "in vitro" addition of theelin to the tissues of spayed rats increases the respiration of anterior pituitary but not of liver or kidney (27). The increase occurs within an hour. Obviously there is a difference in the response of liver and anterior pituitary to theelin in that the anterior pituitary metabolism is directly increased by theelin. The factors which evoke the delayed liver response are still uncertain, and are the subject of the present investigation.

There are indirect reasons for believing that the metabolism of the liver in spayed animals is influenced by both the anterior pituitary and thyroid. Spaying depresses the weight of the thyroid, adrenals and, for a time, the anterior pituitary (2). Amniotin administration restores the oestrus picture within 1 day in the pituitary and after 3 days in the thyroid (1). This suggests that either or both of these glands may influence the metabolic response of the liver to theelin.

The rôles of the anterior pituitary and thyroid in the metabolic change produced by theelin in the liver of spayed rats have been studied in two ways. First, to determine which glands were concerned with the metabolic response, amniotin or theelin was administered to spayed-thyroidectomized or to hypophysectomized rats. Since both glands were found to be essential for the metabolic response of the liver to theelin administration, a second set of experiments was designed to find out which was the first gland stimulated by theelin. This was established by observing the time intervals between thyroxin or anterior pituitary extract injection and the increase in liver metabolism of spayed rats. As stated above, there is a lag period between the increase of the anterior pituitary and liver metabolism of about 66 hours when theelin is injected into spayed rats (3, 27). If the time interval between the administration of thyroxin or anterior pituitary extract and the increased liver metabolism were the same, then both glands must have been stimulated simultaneously by

theelin activity. On the other hand, a difference in lag period of a day in the liver response to these substances, would indicate the sequence of glandular activity evoked by theelin; the substance with the longer lag period would be the first substance secreted. In other words, if anterior hypophyseal extract required 3 and thyroxin only 2 days for stimulating liver respiration, it would be obvious that thyroid secretion must have begun a day later than the anterior pituitary.

Effect of theelin or amniotin injection on liver and kidney metabolism of spayed-thyroidectomized or hypophysectomized rats. Two series of amniotin or theelin injected rats were studied. One consisted of rats spayed at about 60 days and thyroidectomized 1 month later; the other series were hypophysectomized by parapharyngeal route (21) at 90 days. We are indebted to Dr. P. E. Smith for several of the hypophysectomized animals. Spaying the hypophysectomized rats was inadvisable for two reasons; first, the animals did not stand the second operation well, whether it was ovariectomy or hypophysectomy; second, hypophysectomy results in extreme atrophy of the ovaries (22) so that they are functionally inactive. The spayed-thyroidectomized and the hypophysectomized rats were injected with 30 r.u. of amniotin (Squibb) in ethylene glycol[1] or theelin in aqueous solution prepared as described elsewhere (27). The amniotin was kindly supplied by Dr. J. A. Morrell. We are indebted to Dr. Oskar Wintersteiner for the crystalline theelin. The rats were injected about 1 month after thyroidectomy and 2 weeks after hypophysectomy and were 100 to 130 days old. The tissue respiratory measurements were made with differential volumeters previously described (28). All the animals were examined for remnants of thyroid or pituitary, or evidence of pyogenic infection in the middle ears or lungs. In the results summarized in the tables, infected animals or those in which there were any visible glandular remnants, are excluded.

Table 1 presents the mean respiratory rates and quotients of liver and kidney of spayed-thyroidectomized and of hypophysectomized rats before and at various intervals after theelin or amniotin injection. Table 2 is a statistical analysis of the differences observed. No significant changes were produced in these tissues 2, 3, or 4 days after the administration of the hormone, in spite of the presence of oestrus, as judged by the characteristic vaginal smear of nucleated and cornified epithelial cells, on the third day. The experiments clearly show that both the hypophysis and the thyroid are essential for the metabolic effect of theelin on liver tissue.

[1] It is of interest that the intraperitoneal injection of ethylene glycol substrate produced no metabolic changes in liver or kidney as judged by the unaltered respiratory rates and quotients after its administration. The recent findings of H. D. Kesten, M. G. Mulinos and L. Pomerantz (J. A. M. A. **109**: 1509, 1937) relating to the liver and renal lesions produced by diethylene glycol do not apply to the present study.

TABLE 1

Metabolism of liver and renal cortex before and at various intervals after injection of 30 r.u. of theelin or amniotin

DAY AFTER INJECTION	NO. RATS	LIVER				KIDNEY			
		O_2 Consumption cmm./gm./min.		R.Q.		O_2 Consumption cmm./gm./min.		R.Q.	
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Spayed and thyroidectomized rats									
0	10	12.3±0.29	1.3	0.745±0.01	0.044	45.0±1.85	8.3	0.90 ±0.019	0.086
2	8	11.6±0.33	1.3	0.738±0.007	0.027	50.9±1.25	4.9	0.91 ±0.009	0.032
3	6	11.9±1.07	3.6	0.73 ±0.013	0.044	43.4±1.96	5.9	0.88 ±0.033	0.085
4	9	12.6±0.59	2.5	0.783±0.022	0.092	49.6±0.50	2.0	0.947±0.014	0.055
Hypophysectomized rats									
0	6	10.3±0.57	1.9	0.716±0.020	0.067	35.9±1.35	4.5	0.835±0.007	0.022
2	6	10.6±0.65	1.94	0.678±0.034	0.101	38.3±2.2	7.5	0.852±0.022	0.073
3	7	11.9±0.45	1.66	0.676±0.020	0.074	43.2±1.63	6.0	0.783±0.028	0.101
4	5	12.0±0.30	0.89	0.700±0.016	0.048	40.3±2.9	8.6	0.832±0.020	0.059

TABLE 2

Mean differences and their probable errors of liver and renal cortex metabolism before and at various intervals after injection of 30 r.u. of theelin or amniotin

COMPARISON WITH UNTREATED RATS	LIVER				KIDNEY			
	O_2 Consumption cmm./gm./min.		R.Q.		O_2 Consumption cmm./gm./min.		R.Q.	
	Diff.	$\frac{\text{Diff.}}{\text{PE Diff.}}$	Diff.	$\frac{\text{Diff.}}{\text{PE Diff.}}$	Diff.	$\frac{\text{Diff.}}{\text{PE Diff.}}$	Diff.	$\frac{\text{Diff.}}{\text{PE Diff.}}$
Spayed and thyroidectomized rats								
day								
2	−0.7±0.44	1.6	−0.007±0.012	0.6	5.9±2.23	2.6	0.010±0.021	0.5
3	−0.4±1.11	0.4	−0.015±0.016	1.0	−1.6±2.76	0.6	−0.020±0.038	0.5
4	0.3±0.65	0.5	0.038±0.024	1.6	4.6±1.91	2.4	0.047±0.024	2.0
Hypophysectomized rats								
2	0.3±0.86	0.4	0.038±0.039	1.0	2.4±2.58	1.1	0.017±0.023	1.3
3	1.6±0.69	2.3	0.040±0.028	1.4	7.3±2.14	3.4	0.052±0.029	1.8
4	1.7±0.65	2.6	0.016±0.026	0.6	5.6±3.2	1.8	0.003±0.021	0.1

Furthermore, the metabolic change in the liver of spayed animals is merely coincident with, but not an essential change in oestrus.

The effect of thyroxin on liver, kidney and anterior pituitary metabolism of

spayed rats. The action of thyroxin and anterior pituitary thyrotropic hormone on the liver and kidney metabolism of spayed rats was next investigated. It has been found by Foster et al. (12) that l-thyroxin is the active form of thyroxin. The l-thyroxin, $(\alpha) = -4.4$, was isolated from fresh glands and crystallized by Dr. G. L. Foster. We are indebted to him for this substance. It was administered in aqueous solution as previously described (27). The dosage employed in these experiments was 0.03 mgm. per rat which weighed about 200 grams. A 40 per cent increase in B.M.R. of guinea pigs has been produced with a dose of 0.02 mgm. per 100 grams of body weight (12). The respiratory rate of the anterior pituitary was measured with a respirometer previously described (26). Table 3 is a summary of results obtained following the intraperi-

TABLE 3

Effect of intraperitoneal injection of 30γ of levo-thyroxin on tissue metabolism of spayed rats

(O_2 consumption—cmm./gm./min.)
Significant difference italicized

DAYS AFTER INJECTION	NO. RATS	LIVER		KIDNEY		ANTERIOR PITUITARY	
		Mean	S.D.	Mean	S.D.	Mean	S.D.
0	9	13.3±0.36	1.7	49.6±1.25	5.6	10.8±0.20	1.0
1	7	13.0±0.35	1.3	50.3±2.01	7.4	10.0±0.79	2.9
2	7	16.3±0.62	2.3	53.5±1.37	5.0	10.9±0.73	2.7

Mean differences in tissue metabolism after thyroxin injection in spayed rats

COMPARISON WITH UNTREATED RATS	DIFF.	DIFF. / PE. DIFF.	DIFF.	DIFF. / PE. DIFF.	DIFF.	DIFF. / PE. DIFF.
day						
1	−0.3±0.5	0.6	0.7±2.36	0.3	−0.8±0.81	1.0
2	*3.0±0.71*	4.2	3.9±1.85	2.1	0.1±0.75	0.1

toneal injection of the hormone. A significant increase in liver respiration occurred 2 days after the injection of the hormone. No significant changes were observed in the kidney or anterior pituitary at this time.

The increase in liver respiration was 23 per cent on the second day. Although an increase in renal respiratory rate of 8 per cent was found at this time, this was not statistically significant. The difference in this sensitivity of the metabolic change in the liver as compared with the kidney confirms the findings of MacEachern (16). The latent period, then, between thyroxin injection and the metabolic increase of the liver is 2 days. In agreement with our findings are those of Gaddum (13) who found a latent period in the increase in basal metabolism of about 2 days. It is independent of the magnitude of the initial dose (14). On the other

hand Ebina (10) reports that thyroxin administration increases the oxygen consumption of liver in 3 to 5 hours and of kidney after 1 day. Anselmino, Eichler and Schlossman (5) found a very slight increase in the respiratory rate of rat liver and kidney 38 hours after the injection of 1 mgm. of thyroxin (Roche). The difference in magnitude of the response in our experiments as compared with that of Anselmino et al. (5) may be explained by the thyroid atrophy in our spayed animals which may render the animals more sensitive to thyroxin. It has been found (24) that thyroidectomy increases the sensitivity of rats to thyroid administration.

TABLE 4

Effect of intraperitoneal injection of 100 mgm. thyrotropic hormone into spayed rats on tissue metabolism

DAYS AFTER INJECTION	NO. RATS	LIVER				KIDNEY				ANT. PITUITARY	
		O_2 Consumption cmm./gm./min.		R.Q.		O_2 Consumption cmm./gm./min.		R.Q.		O_2 Consumption cmm./gm./min.	
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
0	11	13.3±0.36	1.7	0.774±0.012	0.05	49.6±1.25	5.6	0.923±0.016	0.07	10.8±0.20	1.0
1	11	13.6±0.43	2.0	0.696±0.015	0.07	56.9±1.35	6.4	0.916±0.009	0.04	10.0±0.55	2.6
2	14	11.6±0.28	1.5	0.771±0.012	0.07	51.2±0.40	2.1	0.912±0.010	0.05	10.7±0.57	3.1
3	12	16.6±0.68	3.4	0.748±0.008	0.04	59.0±1.37	5.8	0.864±0.021	0.09		

Mean differences in tissue metabolism after administration of thyrotropic hormone to spayed rats

(Significant differences italicised)

COMPARISON WITH UNTREATED RATS	DIFF.	DIFF./P.E. DIFF.	DIFF.	DIFF./P.E. DIFF.	DIFF.	DIFF./P.E. DIFF.	DIFF.	DIFF./P.E. DIFF.	DIFF.	DIFF./P.E. DIFF.
day										
1	0.3±0.55	1.8	*−0.078±0.019*	4.1	*7.5±1.84*	4.0	−0.007±0.018	0.4	−0.8±0.58	1.4
2	−1.7±0.46	3.7	−0.003±0.017	0.2	1.6±1.31	1.2	−0.011±0.019	0.6	−0.1±0.60	0.2
3	*3.3±0.77*	4.3	−0.026±0.014	1.9	*9.4±1.87*	5.0	−0.059±0.026	2.3		

The effects of anterior pituitary extract on liver and kidney metabolism of spayed rats. Thyrotropic hormone was prepared by flavinate precipitation of acetone dried beef pituitaries according to the method of Dr. Karl Meyer. Its thyrotropic activity has been studied by Werner (29) and Smelser (20). We are indebted to Drs. G. L. Foster, S. C. Werner and G. K. Smelser for supplying this preparation. The activity of the material was such that a portion equivalent to 25 mgm. of dried beef pituitary produced a statistically significant increase in the weight of the guinea pig thyroid in 5 days. The dose injected into the spayed rats was equivalent to 100 mgm. of dried beef pituitary. Table 4 summarizes the results of

these injections. A significant increase in liver and kidney respiratory rates was found 3 days after injection. An unexpected finding was the depression in liver R.Q. and increase in renal respiratory rate 1 day after the injection of the material. These effects were reversed on the second day. Whether this qualitative change in liver and quantitative change in renal metabolism on the first day is due to the action of the extract, which contains gonadotropic, thyrotropic, adrenotropic, and growth promoting factors (23) or to the flavianic acid has not been determined. It was observed that the day after the injection of the extract the peritoneal cavity was stained yellow; this disappeared the next day. The pubic hair was bright yellow the first day after injection and became paler thereafter. Another possibility is that the ketogenic action of anterior pituitary extract (7) in rats, which manifests itself for the first 24 hours after the injection, is effective by increasing the incomplete oxidation of fats in the liver. This possibility is more than remote since it has been found (17) that anterior pituitary extracts do not increase ketone body formation in hepatectomized rabbits. At least two findings militate against the assumption that the altered liver and renal metabolism, one day after the administration of the flavinate extract, are due to thyrotropic action. First, thyroxin administration produces no comparable change, for it increases liver metabolism more than renal metabolism (see above) (16). Second, when the increased metabolism of liver and kidney appear on the third day after administration of the pituitary extract, there are no qualitative changes in the metabolism of these tissues, as indicated by the unaltered R.Q. Thyroid feeding in rats likewise produces no change in R.Q. (9).

It is impossible to draw conclusions from comparison of the data in the literature concerning the metabolic effects of anterior pituitary extracts on basal or isolated tissue metabolism. The reports have been too varied and the results have been obtained with too many different preparations. Although no statement may be made without finding exceptions in the literature, it appears that anterior lobe extract injection stimulates the basal metabolism with about the same lag period as thyroxin. The lag period in the rat following thyroxin administration is about 2 days (13) and about 3 days after anterior pituitary injection (6); in the guinea pig it is the same for both (25), namely, 20 hours. Liver glycogen is decreased in the thyroid treated rat in 2 to 3 days (8) and in the guinea pig in 2 to 3 (15) or 4 days (11). The changes in liver metabolism in the spayed rat show that this metabolic effect of thyrotropic action of the anterior pituitary has a lag period of 3 days.

Discussion. It is apparent from the above observations that both the pituitary and thyroid are intermediate factors concerned with the increased liver metabolism following theelin administration, since the rise

in liver metabolism does not occur in the absence of either one of these glands.

The lag periods following thyroxin, anterior pituitary extract and theelin injections are 2, 3, and 3 days, respectively. These intervals indicate that theelin first stimulates the pituitary, and anterior lobe secretion in turn stimulates the thryoid. It has been found that the injection of theelin produces physiological changes in the anterior pituitary "in vitro" within an hour and "in vivo" in 6 hours and cytological changes in 10 hours (30). Theelin also produces cytological changes in the atrophic thyroid of spayed rats in 24 hours (1). Anterior lobe extracts produce physiological changes in the dog thyroid within an hour "in vitro" (4) and morphological changes in 1 to 4 hours "in vivo" and "in vitro" (18, 19). The lag period in the increased metabolism of liver after anterior lobe administration appears to be due more to the lag period in the action of the substance (thyroxin) secreted by the thyroid rather than to a lag in the action of anterior lobe substance. From this it follows that the thyroid secretes its metabolic stimulant 2 days before the tissue metabolism is increased or within a day after the theelin or anterior pituitary hormones are injected.

The sequence of events leading to increased liver metabolism after intraperitoneal injection of theelin in spayed rats appears to be as follows. Theelin stimulates anterior pituitary respiration in 6 hours (27). Anterior lobe secretion as judged by degranulation of basophils occurs within 10 hours (30). The thyroid responds to anterior lobe secretion within 24 hours of theelin administration, by secreting thyroxin which increases liver metabolism 48 hours after thyroxin secretion and 72 hours after theelin injection.

SUMMARY AND CONCLUSION

Theelin administration in spayed rats increases the metabolism of the anterior pituitary in 6 (27) and the liver in 72 hours (3). This effect on the liver is abolished by hypophysectomy or thyroidectomy. Thyroxin injection increases the liver metabolism of spayed rats in 48 hours; anterior pituitary extract increases it in 72 hours.

Thyroxin injection in spayed rats produces no significant change in kidney or anterior pituitary metabolism at the time when the liver metabolism is increased.

Anterior pituitary extract decreases the liver R.Q. and increases the renal rate of oxygen consumption 24 hours after its administration to spayed rats. The lower liver R.Q. at this time is probably related to the ketogenic activity of anterior pituitary extracts (7, 17). At the time when liver and kidney respiration are both increased, namely 72 hours after the injection of the extract, there is no alteration in tissue R.Q. as compared with untreated spayed rats.

It is concluded that the increased metabolism of the liver of spayed rats injected with theelin or amniotin results from the action of theelin on the anterior hypophysis. The pituitary secretion stimulates thyroid activity which results in increased tissue metabolism. The effect of theelin on liver metabolism is mediated through the anterior pituitary and thyroid, whereas its metabolic action on the anterior pituitary is direct.

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FURTHER STUDIES ON THE RÔLE OF PROGESTERONE IN THE INHIBITION OF ESTROUS CYCLES IN THE ALBINO RAT

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In a previous series of experiments Phillips (1937) found the minimum estrus-inhibiting dosage of crystalline progesterone for the albino rat to be 1.5 mgm. per day. At the same time a crude extract of whole ovaries in daily doses assayed to contain as little as 0.5 mgm. of progesterone (and even smaller doses) was found to inhibit estrous cycles in the albino rat. This unexpected result has led to further investigation of this phenomenon. Since Button and Miller (1936) showed that crystalline estrin may in small dosage interrupt estrous cycles in the rat, our first conjecture was that this greater inhibitory potency of the crude extract was due to the estrin present in the crude extract acting in conjunction with progesterone.

The crude extract initially found to inhibit estrus was the third petroleum ether fraction of whole ovaries prepared by Allen and Goetsch (1936, table 1). This fraction was assayed for progesterone by Young (1936) and separately by Allen; it was, however, not assayed for estrin.

For the present experiments whole ovaries were selected and extracted as shown in diagram 1, modified from the method of Allen and Goetsch (1936). This extract (K, diagram 1) was assayed for progesterone on six oöphorectomized rabbits and assayed indirectly for estrin on 18 castrate female rats by a method based on Allen and Meyer's finding (1933) that the petroleum ether extraction of a 35 per cent ethyl alcohol fraction containing progesterone and estrin removed practically all of the progesterone and 5 per cent or less of the estrin from the alcohol fraction. The estrin remaining in the 35 per cent ethyl alcohol (L, diagram 1) was extracted with benzene and the latter fraction (M) assayed for estrin. By this method the estrin content of the petroleum ether fraction (K) was easily computed.

As in previous experiments, mature female albino rats weighing from 150 to 200 grams were selected. These animals were fed calf meal from self feeders and were given lettuce at regular intervals. The estrous cycles of each rat were followed using the vaginal smear method of Long and Evans (1922). Samples were taken from the vagina with a spatula

at about the same time every day, stained with methylene blue, and studied microscopically. After regular normal estrous cycles were established,

DIAGRAM 1

Scheme for extraction of whole ovaries in the preparation of an estrus-inhibiting fraction

A. 16 kgm. of frozen whole ovaries containing corpora lutea, ground fine, extracted with 1.5 vol. methanol for 48 hours. Ovarian residue extracted 3 times with decanted methanol in Bloor extractor

- B. Ovarian residue discarded
- C. Methanol fraction allowed to stand 48 hrs. at −5°C.
  - D. Fatty precipitate discarded
  - E. Supernatant decanted, diluted 1 − 1 with distilled water, extracted 3 times in Doisy column with petroleum ether (dist. 52°–62°C.)
    - F. 35% methanol fraction discarded (most of estrin here)
    - G. Pet. ether fractions 1-2-3 combined. Pet. ether driven off on steam bath; 18 cc. residual brown oil taken up in 70% ethyl alcohol, extracted 3 times with ⅓ vol. pet. ether.
      - H. Pet. ether fraction conc. to ⅓ vol. on steam bath, washed 3 times with 70% ethyl alcohol.
        - J. Pet. ether fraction discarded. Toxic substances here.
        - K. Pet. ether fraction (progesterone here). Pet. ether driven off on steam bath, residual oil taken up in 70 cc. benzene, assayed to contain 1 Int. U. per 0.4 cc.; total yield = 175 Int. U. or 1 Int. U. per 91 gms. whole ovaries. Minimum estrus-inhibiting dose = 0.2 cc. = 0.5 Int. U. (fig. 1, B).
          - M. Benzene fraction (estrin here) benzene driven off on steam bath to total vol. of 70 cc. Assayed to contain 10 Int. U. of estrin per 1.0 cc. This represents about 95% of the estrin in the 70% ethyl alcohol fraction—approximately 5% of the estrin going along with the progesterone into the petroleum ether fraction.
          - N. 35% ethyl alcohol fraction discarded.
      - I. 70% ethyl alcohol fraction—equal vol. water added, extracted 3 times with ⅓ vol. petroleum ether.
        - L. 35% ethyl alcohol fract. extracted 3 times with ⅓ vol. benzene.

the animals were given daily subcutaneous injections of 0.1 cc. sesame oil containing the dosage of crude extract desired. Before the crude extract was used, most of the benzene in which it had been stored was

removed by evaporation. Six control animals received benzene in sesame oil. The dosage of crude extract given each rat was recorded on the basis of progesterone and estrin content. The crystalline materials used for injection were alpha progesterone, as prepared by Allen, and crystalline estrone; both were dissolved in a few drops of benzene and added to the desired volume of sesame oil.

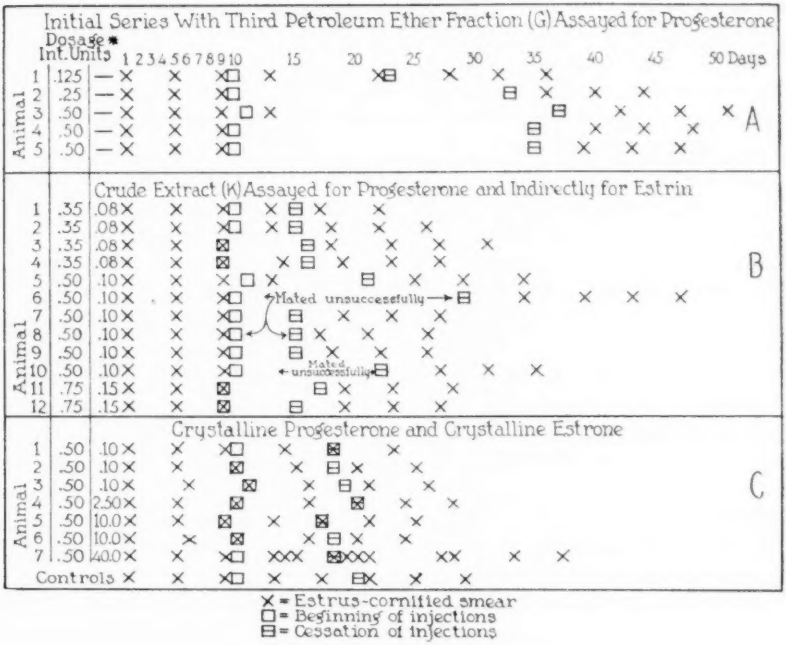

Fig. 1

The results are shown in the figure. In the series already referred to, treated with crude extract, (fig. 1, A) daily doses of the extract containing the equivalent of 0.25 and 0.5 international unit of progesterone inhibited estrous cycles during the period of injection (26 to 31 days) with a prompt return to normal rhythm after discontinuance. The estrin content of this fraction was not known. In the present series of experiments (fig. 1, B) using the crude extract indicated in diagram 1, K, the minimum estrus-inhibiting dosage was found to contain 0.5 international unit of progesterone and 0.1 international unit of estrin. For further evidence

* The second column in the figure indicates the dosage of progesterone in international units; the third column indicates the dosage of estrin in international units. Figure 1, A and B dosages were determined by assay; 1, C dosages were determined by weight of crystalline material.

of inhibition of estrus, animals 6, 8, and 10 (fig. 1, B) were each offered the male daily for many days during the period of inhibition; in no case was the male accepted.

The next step was to combine crystalline progesterone and estrone in amounts equivalent to that contained in the crude extract. Three rats (fig. 1, C) were given daily injections of 0.5 mgm. of crystalline progesterone and 0.1 international unit of crystalline estrin, with no interruption of estrous cycles. Other animals received daily injections of 0.5 mgm. of crystalline progesterone and crystalline estrin in doses ranging from 2.5 to 40 international units. Estrous cycles were slightly prolonged in several cases, but in no case were they inhibited. One animal receiving 0.5 mgm. progesterone and 40 international units of estrin showed prolonged estrus.

From the data presented in this paper it is evident that the above described crude, progestin-containing extract possesses approximately three times the estrus-inhibiting activity of crystalline progesterone. We have shown that the increased potency of the crude extract is not due to the estrin content. We do not believe the inhibiting activity is due to toxicity, since normal cycles were resumed 3 to 5 days after cessation of injections as with the crystalline hormone; the animals, moreover, were without symptoms of illness during and after the period of injection. Either a substance is present in the crude extract which enhances the action of progesterone much the same as palmitic acid enhances the action of testosterone as reported by Miescher, Wettstein and Tschopp (1936), or a substance not progesterone is present which in itself is capable of inhibiting estrous cycles. This may be of interest in connection with the discovery (Allen, 1937, in press) that crystalline progesterone fails to maintain pregnancy in the castrate female rabbit. Further work must be done to identify the nature and action of the substance in question.

SUMMARY

A crude, progestin-containing ovarian extract was found to be three times as effective as crystalline progesterone in inhibiting estrous cycles in the rat; this was not due to the estrin content of this fraction.

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EARLY BLOOD CHANGES DURING AND HYPER-VENTILATION FOLLOWING RESIDENCE AT MODERATE ALTITUDES[1]

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A review of the literature concerning blood changes at high altitudes shows that there is an early rapid rise in the erythrocyte count, followed, apparently without a break, by a slower rise as the subject becomes acclimatized. The following observations, however, show that, at least in our experience, at lower altitudes the initial increase of red cell content is quickly followed by a return to normal, while still at the same altitude. Any more permanent increase with acclimatization must follow this phenomenon at a later date.

These observations were made by a party from the Pathology Department, University of Melbourne, during a nine-day residence at a hostel 200 feet from the summit of Mount Hotham, a peak of 6200 feet in the Australian Alps, during the summer (December) of 1931. We took two days to reach our destination by automobile, the first night being spent at Omeo (about 4000 feet). Observations began on the third day. On the whole the weather was comfortably warm throughout our stay. Rather more exercise was taken by the party than was customary at sea-level, but violent exercise was avoided.

Although our primary concern was with certain aspects of gastric physiology, we also made some observations concerning blood chemistry, blood pressure, and pulmonary vital capacity. For reasons connected with these other investigations, the various chemical estimations were not made on the blood as drawn from a vein, but after the blood had been equilibrated with alveolar air. Owing to the redistribution of ions, therefore, the changes described by other observers will be masked. Other changes, however, are of interest.

METHODS. The blood was drawn into a dry *Record* syringe containing sufficient potassium oxalate as anti-coagulant, from the median-basilic vein of the fasting subject at about 9:30 a.m. each day, i.e., at a constant

[1] This work was made possible by the generous financial assistance of the late Mr. A. M. Nicholas, of Melbourne.

time, in order to avoid those diurnal variations recorded by various writers (Dreyer; Shock and Hastings). The blood was then equilibrated with alveolar air, six times, in a separatory funnel of 400 to 500 cc. capacity, centrifuged for 90 minutes, the plasma separated without loss of CO_2, and the following estimated: *a*, CO_2 capacity of plasma and whole blood (Van Slyke); *b*, chloride content of plasma and whole blood (Van Slyke); *c*, red cell volume per cent (hematocrit), and *d*, CO_2 and chloride contents

TABLE 1

SUBJECT AND DATE	R.B.C. PER CENT HEMATOCRIT	CO_2 AS ME/LIT.			$\frac{CO_2\ c}{CO_2\ s}$	CHLORIDE AS ME/LIT.			$\frac{Cl\ c}{Cl\ s}$
		Blood	Plasma	Cells		Blood	Plasma	Cells	
E. F., age 27: Weight 87.3 kilo									
11.27.31	46.6	32.3	36.8	27.0	.73	79.0	98.5	56.6	.57
12.14.31	50.8	33.1	38.2	28.3	.74	78.8	99.2	59.2	.60
*12.16.31	49.0	31.5				79.8			
12.18.31	48.0	30.1	36.2	23.5	.65	79.8	99.5	58.3	.59
12.28.31	47.0	27.7	33.7	20.7	.61	82.2	102.8	59.2	.58
2.22.32	47.0	30.6	36.2	23.8	.66	76.7	97.1	53.5	.55
C. F., age 29: Weight 64.5 kilo									
11.25.31	46.5	33.1	38.2	25.1	.68	80.3	99.0	58.7	.59
12.15.31	48.0	32.5	36.0	29.0	.81	80.7	100.0	59.8	.60
*12.17.31	47.5	31.5	36.4	25.8	.71	80.7	102.1	57.2	.56
12.19.31	46.5	28.6	32.8	23.8	.73	82.3	101.3	60.5	.60
12.29.31	46.5	27.3	33.5	20.3	.60	79.0	101.6	53.0	.52
2.29.32	46.0	28.6	33.5	23.0	.68	77.2	99.5	50.8	.51
F. A., age 43: Weight 76.4 kilo									
11.27.31	48.0	32.8	35.3	30.2	.85	80.8	100.3	59.8	.59
12.14.31	47.8	35.7	40.4	30.4	.76	84.1	100.9	65.8	.65
*12.16.31	48.0	33.1	38.9	27.0	.69	83.6	103.0	62.8	.61
12.18.31	47.5	35.8	36.2	26.1	.72	81.7	103.2	58.2	.56
12.28.31	46.6	29.9	35.5	23.5	.66	82.8	104.2	58.7	.56
2.22.32	50.0	31.8	36.2	26.7	.74	80.3	101.3	59.3	.59

*At 6000 feet.

of red cells, by calculation. Although the blood of each subject was brought into equilibrium with the alveolar air of the same technician on each occasion on which he was examined, it is realized that the partial pressure of alveolar CO_2 falls at 6000 feet, and would therefore be expected to result in an apparently lowered CO_2 capacity of the bloods examined. The effects of this procedure will be considered in their place.

Results. The changes found by us at 6000 feet can be divided into two periods (table 1 and fig. 1). Although there are exceptions and ir-

regularities, some no doubt due to experimental error, we find certain general changes which are beyond the maximum calculated limits of error. These changes, compared with sea-level figures, are: *a*, at first, a rise in all the quantities and concentrations measured, i.e., of red cell volume, the CO_2 and chloride concentrations in whole blood, plasma and red cells,

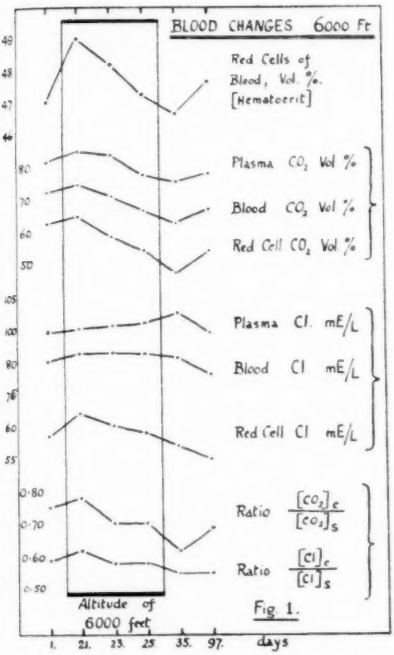

Fig. 1

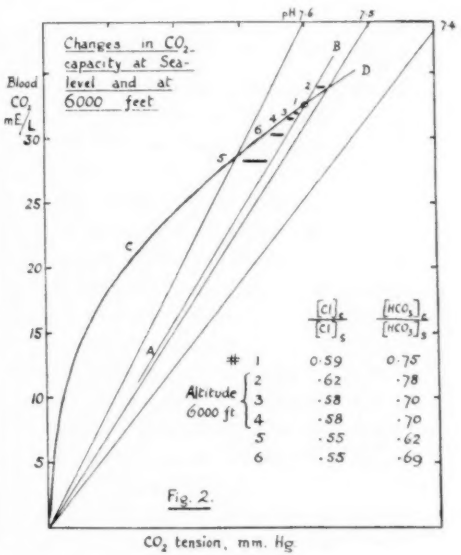

Fig. 2

Fig. 1. Showing the changes in blood chemistry (averages of three individuals) before, during and after a short residence at 6000 feet.

Fig. 2. Showing the changes in CO_2 capacity of the blood before (no. 1), during (nos. 2, 3, 4) and after (nos. 5, 6) a short residence at 6000 feet. (The pH lines were calculated from Van Slyke and Sendroy's graph (13) on the assumption that the alveolar CO_2 tension at sea-level is 40 mm. Hg.)

and in the ratios $\frac{[HCO_3]_c}{[HCO_3]_s}$ and $\frac{[Cl]_c}{[Cl]_s}$, *b*, later, and while still at 6000 feet, there is a progressive fall in all these values (except for a continued rise in plasma chloride) which continue for some time after return to sea-level, even to subnormal levels. Finally, there is a delayed return to normal.

Discussion. A. *The rise and fall of blood CO_2.* In this discussion we shall confine ourselves to the average figures of our three subjects, partly

to neutralize as far as possible experimental errors and partly because all of the subjects have not reacted in a uniform manner, especially as regards their earlier reactions to altitude, a fact which has been noted by other observers. Space, however, does not permit a detailed discussion of each individual.

Changes in the CO_2 capacity of blood can be interpreted only if we know either the pH or the tension of CO_2 with which the blood was equilibrated. Unfortunately, neither of these was determined, so that exact data cannot be presented. We have, however, calculated the ratios $\frac{[HCO_3]_c}{[HCO_3]_s}$ and $\frac{[Cl]_c}{[Cl]_s}$ in each case and we know from the work of Van Slyke and his colleagues (12) that a rise (or fall) in these ratios indicates a fall (or rise) in pH. We are, therefore, in a position to indicate the general nature of the changes that took place in the blood.

These changes can best be illustrated by making use of Haldane's CO_2 – dissociation curve for blood (7) (fig. 2). If the line A B represents the basal or sea-level pH, all points which lie to the left of the line represent a higher pH, and those to the right a lower pH. Hence, if we place point 1 (representing the first blood CO_2 determination at sea-level) on the line A B, point 2 will fall to the right and points 3, 4, 5, and 6 to the left of the line A B. If there were no change in blood alkali these points would fall along the curve C D (drawn approximately parallel to Haldane's curve). It can be calculated from the graph of Van Slyke and Sendroy (13) that this would imply a change of about 0.1 pH or a lowering of alveolar CO_2 pressure by about 30 per cent for point 5. Since it has been shown by Fitzgerald (5) that alveolar CO_2 even after acclimatization to 6000 feet falls only about 15 per cent, and since a pH change of 0.1 is greater even than those found at much greater altitudes, it seems clear that these points must fall somewhere below the curve C D, but above or to the left of the pH base line A B. The points are therefore represented as short lines falling between the curves A B and C D. If figure 2 now approximately describes the blood on each occasion on which examinations were made, we can say:

a. That during the first day or two at 6000 feet there is little or no hyperventilation since the alveolar CO_2 pressure remained practically unchanged. Later, however, hyper-ventilation appears, continues even after return to sea-level, and only slowly returns to normal.

b. That the blood alkali shows similar changes (in two individuals the first reaction to altitude was an increase). Thereafter blood alkali falls, even after return to sea-level and only slowly recovers.

c. That the continued rise of pH even after return to sea-level shows

that the hyper-ventilation is not only a primary phenomenon at 6000 feet, but continues as a primary phenomenon even after return to sea-level, and is not—as we would expect in this later sea-level phase,—secondary to loss of blood alkali. This suggests that some change—an increased sensitiveness—has taken place in the respiratory center, a change from which recovery is delayed for weeks after return to sea-level. We must not overlook the possibility, however, that the greater summer temperatures experienced in Australia in February might have been a factor in retarding a return to the figures of the early summer of late November.

In general, these results are in keeping with those of Douglas et al. (3), who showed that the alveolar CO_2 tension continues to fall and the alveolar oxygen to rise for many days after reaching high altitudes: of Schneider (8) and others (3) who noted that residence at high altitudes may be followed by hyper-ventilation for weeks after return to sea-level: and of Sundstroem (11) who found that the urinary excretion of base during the first two days was much less than later.

Incidentally, our results might account for the fact that some authors have described a shift to the right (or fall) in the CO_2—dissociation curve of blood at altitudes, while others find a shift to the left (or rise) and still others find no change. A glance at our figures shows that each of these changes can be found at different times, i.e., a shift to the left in the early phases and the reverse in the later phases.

B. *The changes in red cell volumes (hematocrit).* The early initial rise in red cell volume has, of course, been noted in every expedition to high altitudes. Gregg et al. (6) showed that it even begins within an hour or less in rapid ascents. Bürker et al. (2), working also at 6000 feet, found an increase of red cells of from 4 to 11.5 per cent and of hemoglobin up to 7 to 10 per cent. Individual differences of reaction found by the Anglo-American (3) and Bürker expeditions are also seen in our results.

Although it is certain that the later and more permanent red cell increase of acclimatization to high altitudes is brought about by a new formation of red cells with an increase in blood volume (Douglas et al., 3; Barcroft, 1) the cause of the early red cell increase is uncertain. Schneider (9) reviewing the literature up to 1921, apparently believes the increase to be due to a concentration of blood, brought about by hyperpnea, loss of CO_2, rise of blood pH and readjustment of pH by loss of alkali and water, partly by the kidneys but chiefly into the tissues. Barcroft (1), on the other hand, favors contraction of the spleen, as part of a general emergency mechanism, with the forcing of red cells out into the blood stream.

Our evidence indicates the reverse of the relationship suggested by Schneider, i.e., there is a rise of red cell volume at the time when there is

no increase of ventilation, while later, red cell volume actually falls coincidentally with the period of over-ventilation and loss of CO_2. When the figures for each individual are examined the same lack of relationship is found. In brief, the rise and fall of red cell volume is due to some mechanism other than water shift. To this extent our results favor Barcroft's views. Such an increase of cell content, by relatively diminishing the volume of alkali-rich plasma, tends to lower the CO_2—dissociation values and thus to mask the changes actually found by us.

SUMMARY

1. At the moderate altitude of 6000 feet there is an early increase in red cell content (hematocrit) which, however, soon returns to normal while still at the same altitude. Similar changes are found in blood CO_2, blood chlorides and the ratios $\frac{[Cl]_c}{[Cl]_s}$ and $\frac{[HCO_3]_c}{[HCO_3]_s}$. These changes continue for at least a week after return to sea-level and only slowly recover.

2. According to our interpretation of the facts presented:

a. There is little or no hyperventilation during the first day or so, the alveolar CO_2 remaining practically unchanged.

b. During the later days at this altitude there is an increasing primary hyper-ventilation with fall of alveolar CO_2, and loss of blood alkali.

c. After return to sea-level the hyper-ventilation, instead of now becoming secondary to the loss of blood alkali, remains primary, with continued loss of blood alkali, suggesting that the primary change is an increased sensitivity of the respiratory center, a change from which recovery is a slow process.

d. There is no relation between the early increase in red cell content and any water shift between blood and tissues. This supports Barcroft's views that such increase is brought about by transfer of red cells from spleen, liver, etc., to the blood stream.

Note: I wish to acknowledge my indebtedness to Misses J. H. Norris, M.Sc. and M. G. Crabtree, M.Sc., of Melbourne, Australia, for their technical assistance.

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SENSITIZATION OF THE ADRENAL GLAND BY PARTIAL DENERVATION

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Since the demonstration by Meltzer and Auer (1904) that denervated smooth muscle responds more actively than the normal to adrenine, several other investigations (cf. Cannon and Rosenblueth, 1937a, for references) have shown that a variety of effectors become more sensitive to stimulation after denervation. At present, it appears that increased responsiveness may be a property common to all denervated effectors.

Pierce and Gregersen (1937) found that the denervated submaxillary gland of the dog gives greater responses to pilocarpine than the normal gland, but smaller responses than the normal to acetylcholine, the chemical mediator of impulses from the chorda tympani (cf. Feldberg, 1933). Morison and Acheson (unpublished observations) tested the responses of denervated and normal adrenal glands to injections of acetylcholine, but the results were not decisive. These experiments may have been complicated by the possibility that a dose of acetylcholine, threshold or maximal for the normal gland, may be paralytic or beyond the optimal concentration for the denervated side (Cannon and Rosenblueth, 1937b).

Simeone, Cannon and Rosenblueth (1938) recently demonstrated that the partially denervated superior cervical ganglion of the cat is hypersensitive to nerve impulses reaching it through its remaining preganglionic fibers. It was deemed of interest, therefore, to test whether partial denervation of the adrenal gland would likewise sensitize it to nerve impulses.

METHOD. Nine cats were used in these experiments. All surgical procedures were performed aseptically under ether anesthesia. In six animals the right (3 cats) or the left (3 cats) major splanchnic nerve was resected in the chest. In three others the minor splanchnic nerve and the abdominal sympathetic chain from the diaphragm to the pelvis were excised, on the right side in two animals and on the left side in the other. One week later, the superior cervical ganglion on the right or left side was removed to sensitize the corresponding nictitating membrane to adrenine.

The experiments were performed 3 to 6 weeks after the partial denerva-

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tions. Dial anesthesia (Ciba, 0.75 cc. per kilogram intraperitoneally) was used. The partially denervated adrenal gland was stimulated electrically through its intact nerve supply, crushed centrally. On the normal side the nerve fibers corresponding to those previously resected on the opposite side were cut acutely at the time of the experiment and the gland stimulated through its remaining nerves decentralized by crushing. Shielded stimulating electrodes were placed on the major splanchnic within the thorax or on the sympathetic chain and minor splanchnic directly under the diaphragm through an opening as low as possible in the chest. The electrodes were packed and separated from the surrounding tissues by dry cotton. Artificial respiration was maintained to insure adequate aeration.

Maximal stimuli were always used, either faradization through a Harvard coil for varying durations or groups of single maximal condenser discharges at 4 per second. The sensitized nictitating membrane (by denervation 2 to 5 weeks previously) served as an indicator for the secretory responses of the adrenals to electric stimulation. Kymographic records of the isotonic contractions were made with the membranes attached to a writing lever under 4 grams' tension and affording 19-fold magnification. At the end of the experiment the responses of the nictitating membrane in centimeters to adrenalin (Parke, Davis), injected intravenously, were plotted against dosage of the hormone in γ (cf. Rosenblueth, 1932). All injections were made into the femoral vein. With the curve thus constructed it was possible to estimate the quantity of hormone liberated by the adrenals in response to stimulation.

Results. In seven of the nine animals, equivalent stimuli liberated 2 to 8 times (average 3.7 times) more adrenine from the chronically partially denervated adrenal than from the control gland. Figure 1 is a typical instance. It is apparent that, to a similar number of nerve impulses the sensitized gland yielded over twice as much adrenine as on the normal side (cf. fig. 2). Adrenine was not liberated in detectable amounts by fewer than 10 maximal condenser discharges at 4 per second. The most striking differences between the two sides were obtained with groups of 20 to 40 such stimuli or with faradization for 0.5 second or less.

With the exception of one case, faradization for 1.0 to 5.0 seconds led to liberation of approximately equal amounts of adrenine from the two sides (figs. 1 and 2, C and D). In the one exception the acutely denervated (control) gland invariably gave a better response to the stimuli regardless of number. The chronically denervated gland in this animal was unusually small, though otherwise grossly normal in appearance. Its weight was 0.16 gram as compared with 0.24 gram, the weight of the control gland. Whether the consistently better response of the acutely denervated gland in this case was due to the difference in size of the two

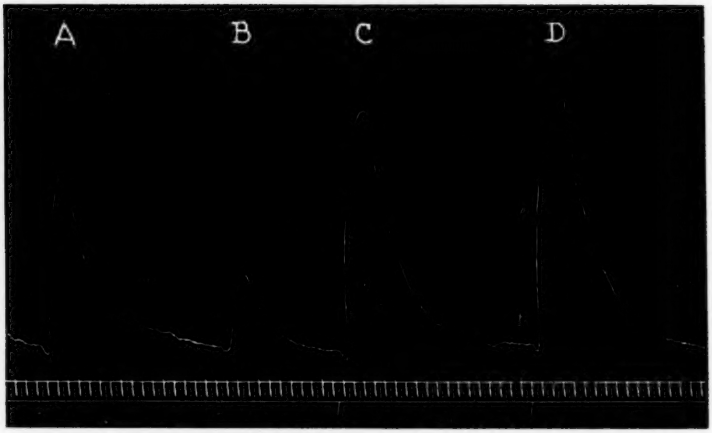

Fig. 1. Cat. Dial (0.75 cc. per kgm. intraperitoneally). Left abdominal sympathetic chain and minor splanchnic resected 4 weeks previously. Same resection on right acutely. Electrodes on right and left major splanchnics. A: response to stimulation of left major splanchnic (20 maximal condenser discharges in 5 sec.). B: response to same number of stimuli applied to right major splanchnic. C and D: responses to faradization of right and left major splanchnics respectively for 1.0 second. Time: 30 seconds. Weight of right adrenal gland: 0.21 gram; of left adrenal gland: 0.19 gram.

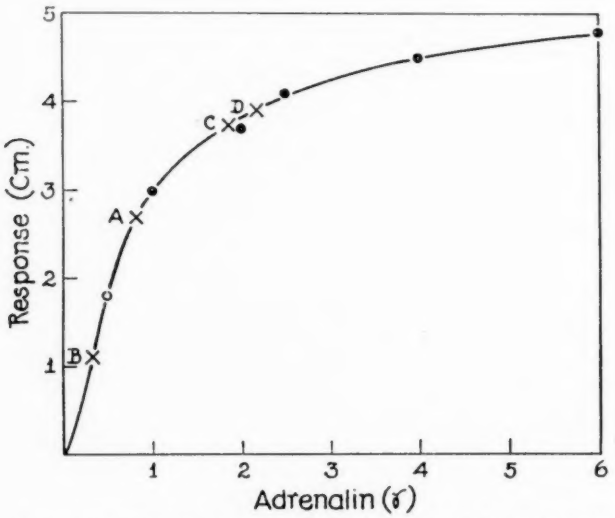

Fig. 2. Curve representing responses (in centimeters) of nictitating membrane of same cat as in figure 1 to graded doses of adrenalin (in γ). A, B, C and D represent points on curve at which corresponding responses from figure 1 fall.

organs or to undetected spread of stimulus on the control side could not be determined.

In another animal the chronically and acutely partially denervated glands gave nearly equal responses to electric stimulation and no explanation was apparent. Since the experiment was done 6 weeks after the denervation it is possible that regeneration may have occurred and the hypersensitivity, that may have been present, was lost (Simeone, 1937). Other experiments, however, done at the same interval had given positive results.

Discussion. The data reported above cannot be attributed to anatomical differences between right and left adrenal glands. Each was used as control in different experiments and, with the two exceptions cited, the chronically denervated gland consistently gave greater responses than the normal. In an extreme instance the response of the denervated gland to similar stimuli was eight times that of the control.

Whether or not the effector cells in the adrenal medulla follow the all-or-none principle is not known. From analogy with sympathetic ganglia, however, it may be assumed that such is the case. The increased response of the partially denervated gland can then be explained either by the activation of a greater number of cells to comparable stimuli than on the normal side or by responses of the same number of cells on the two sides, but repetitively in the chronically denervated gland. These possibilities have been discussed elsewhere in relation to the increased sensitivity of the partially denervated ganglion to nerve impulses (Simeone, Cannon and Rosenblueth, 1938).

The adrenal medulla is histogenetically homologous with the paravertebral sympathetic ganglion. That it can be sensitized to nerve impulses by partial denervation, therefore, is not surprising, since the denervated ganglion is similarly hypersensitive to both acetylcholine and nerve impulses. The adrenal medulla, however, would seem to be unique in that it responds by liberating a powerful hormone into the blood stream. Closer examination of the two cases reveals that the responses of the medullary cells of the adrenal to preganglionic stimulation may be no different from those of postganglionic neurones. Fundamentally, according to the theory of the chemical mediation of nerve impulses (Cannon and Rosenblueth, 1937a), the result of activating either the sympathetic ganglion or the adrenal medulla is the liberation of the precursor of sympathin. The difference lies in the fact that the mediator at the postganglionic nerve endings is probably formed within the effector cells themselves and only the overflow can reach the blood stream, while adrenine is liberated directly into the circulation.

SUMMARY

The responses of the adrenal medulla to nerve impulses are increased by chronic partial denervation (figs. 1 and 2).

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FACTORS AFFECTING THE UPTAKE OF WATER BY FROGS WHEN INJECTED WITH EXTRACT OF THE POSTERIOR HYPOPHYSIS

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The diuretic and antidiuretic effects on the mammalian kidney of extracts of the posterior lobe of the hypophysis are well known. In addition to and probably distinct from this reaction, it has been found recently that injection of such extracts into frogs produces a temporary gain in weight due to the uptake of water. This was demonstrated in 1921 by Brunn (2) and confirmed by Heller (3), Steggerda (9) and Rey (7). The present experiments were designed to establish the significance of factors such as light, temperature, the concentration of salt and pH of the medium on the extent of this reaction.

The general procedure followed was similar to that of Steggerda (10). Frogs of the species *Rana pipiens* were obtained from their natural habitat in southeastern Canada during the month of June and kept in a sloping, galvanized metal tank with a regularly changed supply of water from Lake Ontario and at a temperature of 15–20°C. in the low basement of a stone building. The average weight of 115 unselected frogs from the colony was 22.2 ±8.4 grams (mean ± standard deviation).

Depending upon the variation in results subsequently encountered, from 6 to 36 frogs were used to determine the effect of each variation of each factor listed below and an additional one-third to two-thirds of these numbers were used as controls receiving no injection of extract of the posterior hypophysis. The frogs were placed individually in 400 cc. beakers covered with weighted and numbered wire shields. The beakers contained from 75 to 100 cc. of the watery medium to be studied and the animals were assembled under exactly the same conditions which would prevail the subsequent morning when injections were made. In this manner it was anticipated that the animals would become acclimatized to their altered environment and be in a relatively stable condition when injections were given some 18 to 24 hours later. During this 18 to 24 hour preliminary interval there was no consistent change in the average weight of frogs

placed in distilled water or in dilute hypotonic solutions of sodium chloride although an occasional animal showed a gain or loss of weight as great as 10 per cent. In 24 unselected readings under these conditions, the mean change in weight during the preliminary acclimatizing period was -1.7 ± 3.5 per cent.

Injections were then made into the dorsal lymph sac by a tuberculin syringe of 1 international unit per 10 grams of body weight of Pituitary (posterior lobe) Extract, B.P. 1932,[2] contained in a volume of 0.1 cc. The weight of the injected extract equalled approximately 1 per cent of the body weight. Subsequent weighings were made at intervals of 0.5 to 1 hour or as indicated by the changes encountered.

TABLE 1

Variations in the weight of frogs used as controls, receiving either an inactive injection (72 frogs) or no injection (30 frogs)

Changes in weight are expressed as a percentage of the initial weight

HOURS AFTER INJECTION	RECEIVING INACTIVE INJECTION		RECEIVING NO INJECTION	
	Mean	Standard deviation	Mean	Standard deviation
0.5	+0.4	0.6	0.0	0.4
1.0	+0.5	1.4	+0.2	0.7
1.5	+0.6	1.9	−0.8	1.2
2.0	+0.9	2.6	+0.8	0.9
2.5	+1.0	2.1	−0.4	0.3
3.0	+2.0	3.8	+0.3	0.8
3.5	+1.2	2.6	−0.2	1.1
4.0	+1.5	3.1	+1.0	1.7
5.0	+1.2	4.6	+0.5	1.1
6.0	+2.7	4.8	+0.1	0.8
7.0	+2.1	4.3	+0.9	1.3
8.0	+2.3	3.9	−0.1	0.9

Controls. Two types of controls were employed, one receiving no injection whatsoever and one receiving an equal volume of distilled water or isotonic saline with or without chloretone, tricresol or other preservative. In most of the experiments, the uninjected animals showed no appreciable change in weight over a period of 8 to 12 hours (table 1). The mean changes in 72 animals receiving an "inactive" injection indicated a slight gain in weight during the 8 to 12 hour period, a gain about equivalent to the weight of the injection and largely independent of the salt or preservative content of the injection (at or below isotonicity). There was, how-

[2] Supplies of Pituitary (Posterior Lobe) Extract, B. P. 1932, were generously provided us by the Connaught Laboratories, Toronto, by Charles E. Frosst and Company, Montreal and by Parke, Davis and Company and E. R. Squibb and Sons.

ever, considerable fluctuation in the weight of individual animals. From table 1 it is apparent that variations in weight of from −3 to +6 per cent may be attributable to changes in the weight of the controls. Repeated weighings on individual frogs usually showed a slight consistent gain or a slight consistent loss in weight. In most of the experiments, most of the controls receiving an inactive injection showed a slight gain in weight but except for a few instances there was no consistent change in the average weight beyond the range indicated in table 1. Exceptional behaviour of the controls will be noted subsequently in connection with the experiments concerned.

Species variation. Results obtained with the common green frog, Rana pipiens, were found in preliminary experiments to be more consistent than those with two other species of this genus. The two other species investigated were the large bull-frog, Rana catesbiana and the brown, northern frog, Rana septentrionalis. We are indebted to Prof. Gleb Krotkov of the Department of Biology for indentification of the species. On the average, the greatest response was obtained with Rana catesbiana and the least with Rana septentrionalis, but when the standard deviations were considered it was obvious that no statistically significant difference existed. The coefficient of variation (standard deviation × 100/mean) for the results with Rana septentrionalis was 68, with Rana catesbiana 37 and with Rana pipiens 19, indicating the greater consistency of results obtained with the last named species.

The species Rana temporaria was studied by Rey (7) and Rana clamitans by Steggerda (10) and although statistical data are not provided by these authors, their results were within the range found for the three other species of Rana noted above. It would thus appear that species of the genus Rana respond with a maximal gain in weight of from 10 to 25 per cent following injection of sufficient amounts of extract of the posterior hypophysis and that there is relatively little difference in the response of different species within this genus. On the other hand, Steggerda (10) has noted a response two to four times as great in the toad, Bufo americanus, and a response two-thirds to three quarters less in the mud puppy, Necturus maculatus.

Body weight. Two groups of frogs, one weighing from 13.3 to 19.4 grams (average 16 grams) and the other weighing from 35 to 52 grams (average 44 grams), were used under the same conditions in a medium of distilled water to demonstrate any difference in response with change in body weight. No such difference was encountered, confirming the observations of Rey (7).

The effect of sex. The response of a group of female frogs was found identical to that of a group of male frogs.

Volume of the watery medium. To find if this volume significantly

affected the results, one group of frogs was placed in 50 cc. of distilled water, one in 100 cc. and one in 150 cc. No differences in response were noted with these three volumes of water.

Mode of administration of extract. The same doses of extract of the posterior hypophysis were injected into three groups of frogs in distilled water either intramuscularly, or subcutaneously under the skin of the thigh or into the dorsal lymph sac. The same response was obtained by all three modes of administration.

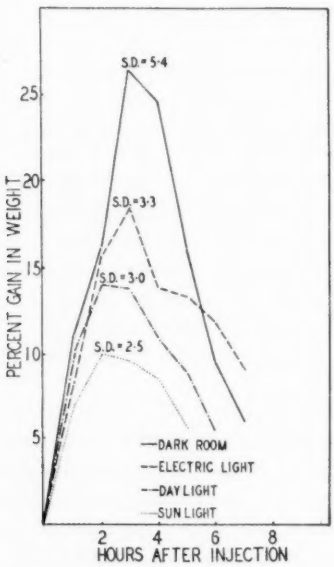

Fig. 1. Mean percentage changes in the weight of frogs receiving injections of extract of the posterior hypophysis under varying light conditions. S.D. = standard deviation.

In another experiment the extract was added to the watery medium in which the frogs were immersed in amounts varying from 1 unit to 10 units per 10 grams body weight. No response whatsoever was obtained by this mode of administration with the doses employed. Steggerda (9) found no increase in the weight of the gastrocnemius muscle of frogs when placed in a medium containing an extract of the posterior hypophysis but there occurred an increase in muscle weight of 15.1 to 27.1 per cent when the extract was injected into the dorsal lymph sac and the muscle removed and weighed 1.5 hours later.

Intensity of light. Four groups of frogs were assembled in distilled water

at room temperature in *a*, the dark room (using red light for weighings); *b*, a windowless room illuminated weakly by an electric lamp; *c*, in the laboratory with ordinary daylight but not in direct sunlight, and *d*, in direct sunlight through glass windows. The mean maximal increases in weight under these conditions were respectively 26.3, 18.5, 14.0 and 10 per cent. The standard deviations of the mean maximal increases in weight are shown in figure 1 and demonstrate that light has a significant depressing effect on the uptake of water by frogs injected with extract of the posterior hypophysis.

Why light should have this depressant effect is unknown. The reaction may possibly be related to the melanophores. But Oldham (6) has found that there is no relation between skin permeability and melanophore dilatation.

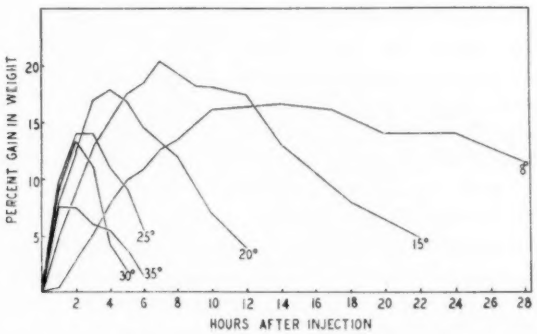

Fig. 2. Mean percentage changes in the weight of frogs receiving injections of extract of the posterior hypophysis under varying temperatures.

The effect of temperature. Six groups of frogs were assembled in distilled water at temperatures varying between 8°C. and 35°C. and the results obtained are shown in figure 2. Groups of six animals each were assembled in galvanized metal tray supports containing the individual beakers and the whole was partially immersed in water in large pans. A thermometer was incorporated into the metal supports and the temperature was regularly recorded and kept within ±1°C. of that indicated on figure 2. In each case, the experiment was assembled 18 to 24 hours before injection of extract was made.

In this experiment certain changes were recorded in the weight of the controls. The frogs withstood a temperature of 35°C. poorly and a few died. No significant changes were recorded during the course of the experiment in the weight of animals maintained at temperatures from 15°C. to 35°C. In the frogs kept at 8°C., a progressive, consistent increase in

weight of controls began to appear at 10 to 12 hours (mean increase at this time 4.6 per cent) rising to a mean increase of 13.5 per cent at 24 hours after which the curve flattened out. This latter was an interesting observation which would have a bearing on the physiology of hibernation in frogs.

The effect of injecting posterior pituitary extract in these animals is shown in figure 2. Lowering the temperature of the animals was found to prolong the response and also, within certain limits, to increase the extent of the gain in weight. There was a statistically significant difference in the mean maximal increases in weight between animals maintained at 20°C. or lower and those maintained at 35°C. From figure 2 it is obvious that the response at higher temperatures is brief and slight and at lower temperatures is prolonged and somewhat greater.

The duration of the reaction at 25°C., 20°C. and at 15°C. by comparison suggests that a decrease of approximately 5°C. prolongs the reaction for approximately double the time. Since the body temperature of frogs closely follows that of their environment, these temperatures may be considered to have existed within the bodies of these animals. The relationship of response to body temperature indicates that at some point in the reaction, between the injection of the hormone and the subsequent uptake of water, there is a chemical process involved the rate of reaction of which doubles for each increase of 5°C.

The latter conclusion brings to the fore the question, what is the mechanism of the water imbibition induced in frogs by extracts of the posterior hypophysis? Steggerda (9) has shown that the relative uptake of water by the muscles of injected frogs is little greater than that of the whole animal. Thus water must be taken up by many tissues other than muscles, and some of these, such as the connective tissue of the skin (5), have been found to take part in the reaction. Ligating the cloaca and thereby preventing the escape of formed urine results in a much greater increase in weight of injected frogs, but the surplus gain is equal to that seen in the uninjected controls, which evidence suggests that antidiuresis is probably not a factor of moment in the water uptake of injected frogs (9). When skinless frogs are injected there is no increase in weight indicating that the skin is of vital importance in the mechanism (9). Heller (3) found that decerebration inhibited the gain in weight of injected frogs but Steggerda and Freedman (11) were unable to confirm this and further noted, confirming Adolph (1), that it did affect water retention by the controls receiving no hormone. Rey (8) found total hypophysectomy or injury to the tuber cinereum without effect on the water uptake of injected frogs. From the evidence available, it would thus appear that the reaction in injected frogs is due to water absorption through the skin which may or may not be augmented by some antidiuresis, followed by storage of such

water in muscles, subcutaneous and other tissues; that it is probably not affected by decerebration of other injury to the brain as a whole or in parts and that a chemical process enters at some stage.

The concentration of salt in the frog bath. Heller (3) recorded a few observations on the effect of sodium chloride in the solution bathing the frogs and Adolph (1) noted variations in the permeability of the skin of frogs to water containing varying amounts of salt. In the present experiments, varying concentrations of sodium chloride, potassium chloride and sodium phosphates were added to the bath and the results obtained are

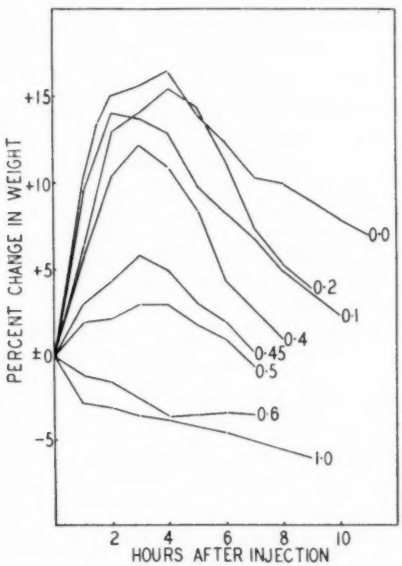

Fig. 3. The effect of injection of extract of the posterior hypophysis on the mean weight of frogs in distilled water containing varying percentages of sodium chloride.

summarized in figure 3. No significant differences were noted in the weight increase of injected frogs on adding sodium chloride to a concentration as great as 0.4 per cent. A concentration of this salt in excess of 0.4 per cent definitely inhibited the reaction and in 0.6 per cent strengths and over there was actually a weight loss which also appeared in the controls receiving an inactive injection.

Similar experiments were performed with potassium chloride. Sodium dihydrogen and sodium monohydrogen phosphates were also added to the bath in proportions necessary to make a final solution of pH 7.0. The results obtained with these further salts were practically identical to those

with sodium chloride. This evidence would suggest, although it does not necessarily prove, that the effect of the several salts studied was entirely physical, presumably by increasing the energy required to draw water into the body through the skin from a medium containing salt in solution.

The pH of the bath. Solutions of the primary and secondary sodium and/or potassium phosphates were made and combined in varying proportions to give a final solution containing 0.3 per cent of salt and a pH ranging from 5.8 to 7.9. In a 0.1 per cent concentration of phosphates the

TABLE 2

The effect of pH of the medium on the increase in weight of frogs injected with extracts of the posterior hypophysis

pH	MEAN MAXIMAL INCREASE IN WEIGHT	STANDARD DEVIATION OF MEAN	HOURS AFTER INJECTION
	per cent		
5.8	9.2	2.7	2.5
6.2	11.1	1.1	2.5
6.5	12.9	2.5	3.5
6.8	14.2	7.5	3.5
7.0	16.2	4.1	3.5
7.7	12.6	4.9	3.5
7.9	10.9	3.5	2.5

TABLE 3

Increase in the weight of frogs following varying doses of extract of the posterior hypophysis contained in the same volume of injection

DOSE	MEAN MAXIMAL INCREASE IN WEIGHT	STANDARD DEVIATION OF MEAN	HOURS AFTER INJECTION
units/10 gm. body weight	*per cent*		
1.0	14.4	3.4	3.0
0.50	14.3	5.7	2.45
0.25	13.7	5.7	2.25
0.175	8.6	2.5	2.25
0.10	7.5	2.9	2.00
0.050	6.6	3.8	1.15
0.025	2.3	2.5	1.00

medium tended to become acid on standing in contact with frogs but a 0.3 per cent solution provided sufficient buffering capacity to prevent any appreciable change in pH during the course of the experiment. The results of this experiment have been summarized in table 2. In these experiments, as in those previous, the greater the maximal increase in weight, the longer did the reaction last. It is obvious from table 2 that the optimum pH producing the greatest reaction was pH 7.0 but that from pH 6 to pH 8 there was comparatively little difference in the results.

Relation to dosage. Doses varying from 0.025 to 1.0 international unit per 10 grams body weight were incorporated into the same volume of fluid and injected into groups of frogs with the results shown in table 3. In general the response fell with decrease in the dose below a certain dose which produced the maximal response. The minimal dose of extract producing the maximal response varied slightly from one marketed extract to another.

Assay of marketed extracts. The minimal amount of several marketed extracts of the posterior hypophysis required to produce an uptake of water equal to 10–15 per cent of the body weight was determined and the results are summarized in table 4. Four marketed preparations were found to contain about the same amount of the frog water balance principle. Pitressin, however, contained much less of this principle than Pitocin which is of interest since the reverse is true with respect to the mammalian diuretic-antidiuretic principle. Heller (4) and Steggerda and Essex (12) likewise have found less of the water balance principle of frogs in Pitressin than in Pitocin.

TABLE 4

The minimal amount of various marketed extracts of the posterior hypothesis required to produce an average gain in weight of 10 to 15 per cent when injected into frogs

PREPARATION	UNITS OF PREPARATION CONTAINING MINIMAL AMOUNT
Connaught	0.25
Parke, Davis Pituitrin	0.25
Squibb	0.50
Frosst	0.75
Parke, Davis Pitocin	0.25
Parke, Davis Pitressin	>2.0

SUMMARY

An investigation was made of the effect of several factors on the uptake of water and gain in weight of frogs injected with extracts of the posterior hypophysis.

Factors found to have little or no effect on this reaction were: difference in species within the genus Rana, body weight, sex, volume of water in the frog bath and intramuscular, subcutaneous or dorsal lymph sac modes of injection.

Adding extracts to the water bath containing frogs did not produce a change in weight with the amounts used. The extent of the reaction varied inversely as the intensity of light. Decreasing the temperature prolonged the duration and increased the height of the reaction within certain limits. Concentration of sodium chloride, potassium chloride or

sodium phosphates greater than 0.4 per cent in the frog bath inhibited the reaction. The optimum pH of the bath producing the greatest response was found to be pH 7.0 with little change in response from pH 6 to pH 8. There was a roughly direct relation between response and dosage until a maximum response was obtained after which a larger dose had no further effect. Several marketed extracts of the posterior hypophysis were found to contain roughly the same amount of the frog water balance principle but Pitocin contained much more than Pitressin.

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THE BIOASSAY OF MARE SERUM HORMONE

A Comparison of Ovarian and Uterine Weight Methods

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The gonad-stimulating activity of mare serum during a limited period of pregnancy was first described by Cole and Hart (1). Follicle stimulation, ovulation, corpus luteum formation, and estrus changes in the uterus and vagina, may be produced in immature rats by the injection of the mare serum hormone. Although any one or combination of these effects might be made the basis of assay, it is desirable to choose for this purpose a response which shows the closest relationship to change in dosage. The gonadotropic hormone in pregnant mare serum differs from those found in most other sources in that ovarian weight increases with dosage over an extremely wide range. It is possible to produce in rats approximately a 15 times increase in mean ovarian weight by increasing the dosage of hormone to not more than 15 times the minimum amount necessary to produce a measurable response. Thus it is not surprising that ovarian weight has been most often used in assaying this hormone.

Cole and Saunders (2), Meyer (3), and Evans, Gustus and Simpson (4) have employed assay units based upon a minimum ovarian response in rats or mice. A unit of this magnitude has the advantage of representing approximately a normal maturity change in the ovary. However, for quantitative evaluation it possesses the disadvantage of falling in a range where ovarian weight is not sensitive to rather large changes in dosage.

Levin and Tyndale (5) have found that for the assay of gonad-stimulating substances from castrate urine, a method based on uterine weight is much more accurate than one based upon ovarian weight. In preliminary studies these workers found the uterine weight method applicable to the mare serum hormone but the results were less uniform than with castrate urine.

The purpose of these studies has been to construct assay curves by plotting ovarian and uterine weights at increasing dosages of the mare serum hormone administered to immature rats, thus permitting a direct comparison of the two methods and their respective units. In this way it is possible to establish ovarian and uterine weight units at positions on the curves which will permit a maximum degree of accuracy in assaying the

hormone. An accurate method of standardization is highly desirable since this hormone is now being studied clinically.

EXPERIMENTAL. *Material.* The hormone fraction used in these studies was prepared from pregnant mare plasma by a method which we have previously described (6). The active acetone precipitate contained 8.2 per cent total nitrogen and preliminary tests on 21 to 23 day old rats indicated that a total dosage of 0.05 mgm., divided into 3 daily subcutaneous injections, would produce ovaries approximately 5 times the control weight when autopsy was made 96 hours after the first injection. A stock solution in 10 per cent alcohol was prepared containing 0.5 mgm. of active powder per cc. This solution was ampouled and frozen and used for the preparation of fresh dilutions at the time of each assay. We have previously shown (6) that this type of highly purified product reproduces the characteristic gonadotropic properties of the original crude plasma and thus truly represents the mare serum hormone.

METHODS. The female rats used in these experiments were from our own Wistar strain colony and weighed 35 to 45 grams at 21 to 23 days of age when the first injections were made. At weekly intervals a series of rats were injected covering as far as possible the entire range of dosage using 5 rats on each dose. This was repeated until each dosage point was represented by 10 to 25 animals, the larger numbers being used on the more critical points. Three hundred and ninety-five rats were used in constructing the curves here presented.

Preliminary experiments, in which the time of autopsy was varied from 72 to 120 hours after the first injection, indicated that at 96 hours a maximum effect upon the ovaries was produced. Three groups of 20 rats each were injected with a total dose of 0.10 cc. of stock solution per rat distributed as 3 equal daily subcutaneous doses. Autopsies at 72, 96, and 120 hours after the first injection showed mean ovarian weights of 43, 54, and 49 mgm. respectively. The mean uterine weights were 83, 106, and 113 mgm. respectively. The 96 hour period was chosen for all subsequent experiments reported in this article.

The stock solution after suitable dilution with physiological saline was injected subcutaneously in 1 cc. doses on each of 3 successive days. Ninety-six hours after the first injection the rats were sacrificed and the ovaries dissected free of oviducts and bursae, examined for corpora lutea, and weighed. The uterus was dissected free of its mesentery and freed of intrauterine fluid by pressing between dry filter papers, and weighed. In order to make the best possible use of the material at hand, we also recorded the weights of the kidneys, adrenals, thyroids and hypophyses for the purpose of detecting the possible presence of other hormones capable of influencing these tissues. Evans and co-workers (7) have observed enlargement of the hypophysis in female rats injected with mare serum hormone.

RESULTS. Autopsy data are recorded in table 1. In figure 1 we have plotted the mean ovarian and uterine weights for each dose of the stock solution of mare serum hormone. By using the lower scale in figure 1, the result in rat units can be read off directly for each ovarian weight. The results show that the primary effect of this hormone upon ovarian weight constitutes the most satisfactory basis of assay because it shows the greatest magnitude of response. The maximum mean ovarian weight is more than 15 times that of the controls.

TABLE 1

Autopsy data from rats injected with mare serum hormone

TOTAL DOSE STOCK SOLUTION	NUMBER OF RATS	MEAN BODY WEIGHT AT AUTOPSY	WEIGHT OF TISSUES (MEAN ± E_M*)						OVARIES	
			Ovaries	Uterus	Kidneys	Adrenals	Thyroid	Hypophysis	With CLOv†	With CL†
cc.		*grams*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*per cent*	*per cent*
0.0000	25	46.6	14.5 ±0.4	15.4 ±0.5	630 ±10	11.2 ±0.4	11.2 ±0.3	2.5 ±0.6	0	0
0.0050	15	45.9	13.1 ±0.5	17.3 ±2.3	628 ±18	11.8 ±0.4	10.4 ±0.4	2.7 ±0.9	0	0
0.0075	15	44.7	12.6 ±0.6	66.1 ±8.9	565 ±15	11.9 ±0.4	10.4 ±0.4	2.9 ±0.5	0	0
0.0100	15	44.2	15.2 ±1.4	71.6 ±7.0	594 ±12	12.0 ±0.5	10.3 ±0.4	2.8 ±0.8	27	0
0.0150	15	44.1	17.1 ±1.1	88.5 ±3.4	607 ±16	12.8 ±0.5	10.3 ±0.4	2.8 ±0.7	47	0
0.0200	15	44.9	21.2 ±1.5	88.7 ±2.2	593 ±16	12.3 ±0.4	9.9 ±0.4	2.8 ±0.8	87	0
0.0250	25	45.1	24.4 ±1.3	90.4 ±2.1	618 ±15	13.1 ±0.4	11.0 ±0.3	2.8 ±1.0	92	0
0.0375	15	43.4	23.3 ±1.2	97.1 ±2.7	597 ±16	12.2 ±0.3	9.9 ±0.3	2.8 ±0.9	67	0
0.0500	25	45.1	25.0 ±1.1	111.5 ±4.0	617 ±11	12.1 ±0.3	10.3 ±0.3	2.8 ±1.0	36	0
0.0750	25	44.4	35.0 ±2.5	121.9 ±2.9	628 ±14	11.4 ±0.4	10.0 ±0.5	3.0 ±0.7	12	0
0.1000	25	45.7	58.8 ±3.8	121.8 ±2.8	653 ±15	11.2 ±0.4	10.5 ±0.6	3.1 ±0.7	0	0
0.1250	25	42.5	80.7 ±5.1	106.4 ±2.9	622 ±12	11.8 ±0.4	10.8 ±0.3	3.0 ±0.6	0	24
0.1500	25	42.6	108.9 ±7.0	107.5 ±2.7	632 ±13	11.6 ±0.3	11.3 ±0.4	3.1 ±0.8	0	36
0.1750	25	45.0	144.9 ±8.0	108.4 ±2.6	656 ±14	12.5 ±0.4	10.9 ±0.4	3.1 ±1.0	0	36
0.2000	25	42.2	153.7 ±7.6	105.3 ±1.9	638 ±11	12.6 ±0.4	10.3 ±0.3	3.3 ±0.7	0	56
0.2250	25	42.9	195.8 ±8.0	107.1 ±2.2	625 ±13	12.4 ±0.4	10.9 ±0.4	3.3 ±0.8	0	84
0.2500	25	44.1	206.6 ±7.1	105.5 ±2.5	630 ±14	12.2 ±0.4	11.0 ±0.4	3.4 ±0.6	0	88
0.3000	10	41.6	229.8 ±9.5	101.6 ±1.6	627 ±24	12.1 ±0.4	9.7 ±0.4	3.0 ±0.9	0	60
0.4000	10	42.7	217.9 ±9.8	102.5 ±3.1	650 ±17	11.7 ±0.8	9.7 ±0.5	3.1 ±1.4	0	80
0.6000	10	43.0	212.2 ±13.5	97.8 ±3.1	633 ±24	10.9 ±0.4	9.4 ±0.5	3.0 ±1.4	0	70

* The mean deviation of the mean (E_M) was calculated according to Scott (8).

† CLOv indicates corpora lutea of ovulation; CL indicates corpora lutea atretica.

The data here presented show that a mean ovarian weight of 25 mgm. or less falls on a part of the curve which is not suitable for accurate quantitative assay. However, in that portion of the curve where the mean ovarian weight falls between 35 and 145 mgm., the ovarian response is practically a straight line function of dosage. This is also a region of high sensitivity to small changes in dosage and thus constitutes the most suitable part of the ovarian curve for the quantitative assay of this hormone. Our rat-ovarian unit has been chosen on this part of the curve.

A rat unit may be defined as the minimum total dose of hormone which, administered to 21 to 23 day old rats weighing 35 to 45 grams in 3 equal subcutaneous injections at daily intervals, will produce at autopsy, 96

hours after the first injection, a mean ovarian weight of 65 mgm. which is four to five times that of the controls.

Our results indicate that the uterine weight can also be made the basis of assaying mare serum hormone since it shows a maximum increase to almost 8 times the control weight. The uterine weight increases recorded in table 1 are induced by estrogenic hormones produced in the ovaries as a result of follicle stimulation by the mare serum hormone. The purified hormone preparation used in these experiments is entirely free of estrogenic substances. The uterine weight shows its greatest sensitivity in a

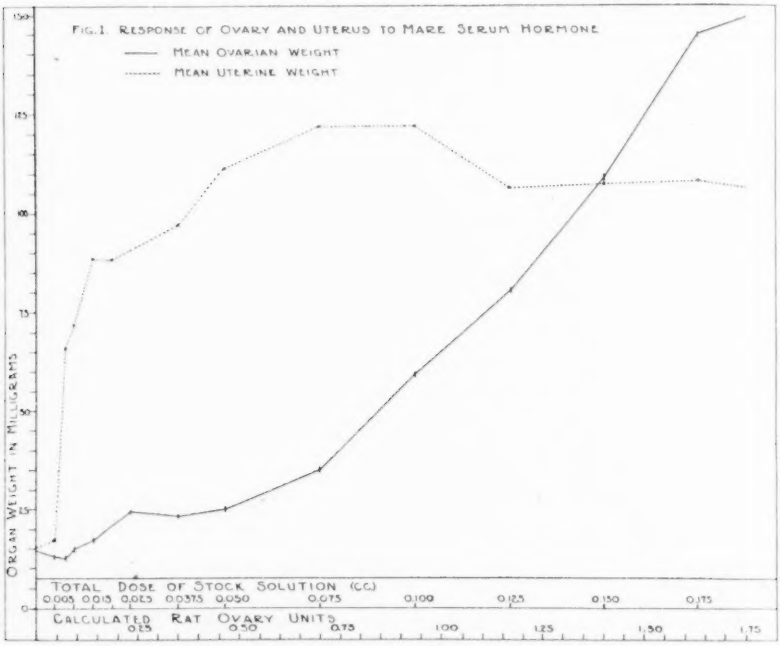

Fig. 1

range of dosage which is well below that necessary to produce an increase in ovarian weight. Thus, the uterus method has the advantage that it is capable of measuring very small amounts of the hormone. A similar advantage was observed by Levin and Tyndale (5) for the mouse uterus method applied to castrate urine.

From table 1 it is seen that 0.005 cc. of hormone stock solution produced no significant effect upon the uterus. However, increasing the dose to 0.0075 cc. produced an increase in mean uterine weight to approximately 4 times that of the controls. Since at higher levels the uterine weight is

much less sensitive to changes in dosage, the practical working part of the uterine curve is extremely narrow. This constitutes a serious disadvantage as compared to the broad range of dosage covered by the ovarian curve. However, for the purpose of comparison with the ovarian weight unit previously described, we have chosen a uterine weight unit falling on the most sensitive part of the uterine curve. This is essentially a threshold unit. A uterine weight unit may be defined as the minimum total dose of hormone which, administered under the conditions described in this article, will produce a 100 to 150 per cent increase in the mean uterine weight.

From figure 1 it is seen that 1 uterine weight unit is represented by 0.006 cc. of our hormone stock solution. The rat ovarian weight unit is represented by 0.107 cc. of our stock solution. Thus, our rat ovarian weight unit is equivalent to approximately 18 uterine weight units.

The kidneys, adrenals and thyroid are not affected by the mare serum hormone under the conditions of our experiments. The slight increase in hypophysis weight might be interpreted as confirming the findings of Evans and co-workers (7). However, the high mean deviation of the mean would indicate that the differences here recorded are probably not significant.

In the last 2 columns of table 1 we have recorded the incidence and type of corpora lutea found in the ovaries at autopsy. It is interesting to note that, for the mare serum hormone, luteinization is a regular function of dosage. At doses in the neighborhood of 0.25 rat ovarian units a majority of the ovaries show corpora lutea of ovulation. These diminish at higher doses until, at approximately 1 unit, not a single corpus luteum was observed in the 25 rats autopsied. At this dosage, under the conditions of our experiments, the mare serum hormone is purely follicle stimulating in the rat. At still larger doses, corpora lutea atretica appear, reaching a maximum percentage above 2 units.

The ovarian weight method here described has been used in our fractionation studies (6) on the mare serum hormone. Our procedure has been to estimate the approximate potency of an unknown preparation by a series of preliminary doses, using 3 to 5 rats on a dose On the basis of the results so obtained, 1 or 2 groups of 10 to 20 rats each are injected with doses estimated to yield 1 unit. The unit dose necessary to produce a 65 mgm. mean ovarian weight is then calculated by interpolation from the table or curve. At this writing more than 4000 rats have been used in assaying and re-assaying fractions covering a wide range of purity. The results have been highly satisfactory.

SUMMARY

Using 395 immature rats, the mean ovarian and uterine weights have been recorded for the mare serum gonadotropic hormone administered

over a wide range of dosage. The assay curves have been constructed. The ovarian weight curve is more satisfactory for quantitative assay although the increase in uterine weight may be useful in detecting small amounts of the mare serum hormone.

The rat ovarian unit is defined as the minimum total dose of hormone which, administered to 21 to 23 day old rats weighing 35 to 45 grams in 3 equal subcutaneous injections at daily intervals, will produce at autopsy, 96 hours after the first injection, a mean ovarian weight of 65 mgm. which is 4 to 5 times that of the controls. This rat unit is approximately 18 times as large as a rat uterine unit based on a 100 to 150 per cent increase in mean uterine weight.

The mean weights have been recorded for kidneys, adrenals, thyroid and hypophysis. No significant effects of the mare serum hormone on these tissues was observed.

Luteinization in the ovaries of immature rats injected with mare serum hormone is a regular function of dosage. Corpora lutea of ovulation appear at small doses; corpora lutea atretica at very high doses. At an intermediate dose of approximately 1 unit, the effect on the rats' ovaries is purely follicle stimulating.

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CHANGES IN THE BLOOD FLOW THROUGH THE BRAIN AND MUSCLES DURING THE ARREST OF BREATHING[1]

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When the lungs of the beaver are inflated the flow of blood through the muscles rapidly decreases and there is at the same time an increase of flow through the brain. The opposite changes in the peripheral circulation seems to prepare the animal for the endurance of asphyxia by conserving the supply of oxygen available at the beginning of asphyxia for the use of the brain (Irving, 1937). I have observed the same adjustment of the circulation in cats, dogs, and rabbits. The vascular change occurs rapidly and regularly and seems to be purposive or anticipatory in nature. It involves a large and diverse vascular field and is set in operation not only by the inflation of the lungs, but by any method of arresting the ventilation.

METHODS. About 90 animals (muskrats, beaver, cats, dogs, and rabbits) have been examined. There was no essential qualitative difference in the vascular responses in the different mammals. The animals were anesthetized with chloralose (0.06 gram per kilo) and urethane (from 0.07 gram per kilo and upward as needed). Blood pressure was recorded in a carotid or femoral artery.

Changes in blood flow in the tissues were recorded simultaneously by two electrical resistance wire flow meters. The instruments operated on the principle of the hot wire anemometer, as previously described (Irving, 1937). Using a resistance wire element with a foot of nickel wire 0.0015 inch in diameter, a current of 150 milliamperes raised its temperature, by measurement, less than two degrees above the tissue. Heat was evolved by the heated wire at about the same rate as by a gram of muscle at rest, and so the temperature and quantity of heat applied to the tissue were not unduly large. The instrument was checked by observing the indication of decreased blood flow upon clamping the arterial blood supply of the tissue. In this test the indicator moved within two seconds of the change in flow. The instrument showed change of flow quickly, but did not show the rate of flow.

The resistance wire needle was inserted into various muscles or into the

[1] This investigation was aided by grants from the Banting Research Foundation, from the Penrose Fund of the American Philosophical Society, and from the Ella Sachs Plotz Foundation.

brain for a distance of one to one and a half centimeters through a trephined opening in the parietal eminence. The movement on the galvanometer scale was transferred by a manually controlled mechanical system to the kymograph paper on which the arterial blood pressure and signals were recorded. In all records of blood flow downward deflection means diminished flow. In order to avoid interference of the recording points, the flow records are displaced from the signal marker position, time and blood flow record. The flow records may be synchronized with blood pressure by comparing the point where rapid change in blood pressure, such as occurred at the start of inflation, caused a rapid change in blood flow.

Fig. 1

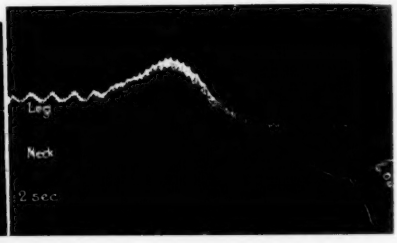

Fig. 2

Fig. 1. Changes in blood flow in normal and denervated (sciatic and femoral and obturator cut) hind leg flexor muscles of a rabbit during inflation of lungs (at pressure of 9 mm. Hg)—left, and when trachea was clamped—right.

Fig. 2. Changes in blood flow during cessation of artificial ventilation in the normal neck and sympathectomized right hind leg flexors of a cat. Cat had last four lumbar and first two sacral autonomic connectors removed, was curarized, and maintained by artificial ventilation. Ventilation was not resumed and fatal asphyxia occurred.

Changes in ventilation which affect the peripheral circulation. When the lungs were inflated with pressures of from 1 to 15 mm. Hg, the flow of blood through the muscles diminished (fig. 1) while it increased through the brain (figs. 4, 5, 6). Whether or not apnea occurred did not affect changes in blood flow, except that vigorous breathing movements mechanically disturbed the recorders and by altering the blood pressure often caused changes in blood flow which obscured the effects of constriction or dilatation.

Clamping the trachea also caused the same vascular response as inflation (fig. 1). The arrest of artificial ventilation in a curarized animal was likewise effective in causing the vascular change (fig. 2). Apparently muscular movements or tone did not cause the vascular change and the only condition common to all experiments was the arrest of ventilation.

The reduction in muscular flow was recorded within 12 seconds in some

cases, and usually within 20 seconds. The increase in cerebral flow was recorded within 15 seconds. Complete arrest of the circulation to a muscle by clamping the artery showed a change in about 2 seconds. The change in the latter case was more extreme, and the delay in recording was less. It is probable that the vascular change followed the arrest of breathing after a slight delay, but not later than by about 12 seconds.

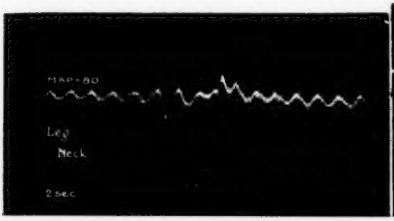

Fig. 3

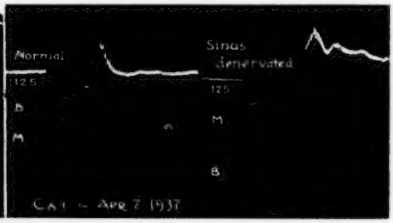

Fig. 4

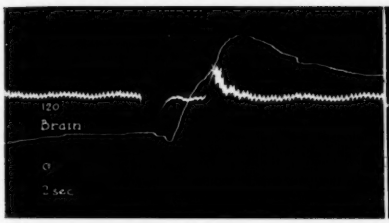

Fig. 5

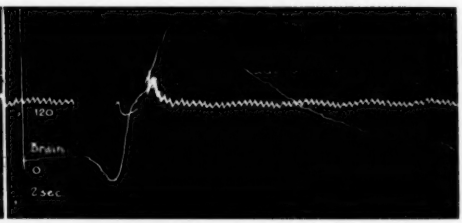

Fig. 6

Fig. 3. Blood flow in normal neck and sympathectomized leg muscles of curarized cat during inflation of the lungs. Blood flow in the sympathectomized leg increased during inflation.

Fig. 4. Blood flow during inflation of the lungs in the brain, *B*, and muscle, *M*, of a cat before and after denervation of the carotid sinus areas, showing the accentuation of vascular changes after denervation of the sinus areas.

Fig. 5. Blood flow in the brain of a normal cat during inflation of the lungs.

Fig. 6. Blood flow during inflation in the brain of a cat after cutting cervical sympathetics. Compare with figure 5.

The effects of mechanical pressure changes. It was observed that arterial pressure usually fell during artificial inflation of the lungs in proportion as the pressure of inflation increased. The immediate effect of the diminished arterial pressure in reducing muscular flow is shown in figure 1. As the arterial pressure subsequently rose, blood flow was partially restored, but the muscular flow continued to diminish as the pressure increased. Flow through the brain was also passively decreased by the

fall in pressure, but the flow increased and usually exceeded the original value before the blood pressure was restored (fig. 4). Often only slight inflation was required to produce apnea, and in a quiet animal the arterial pressure was not changed and the opposite changes in blood flow appeared more plainly.

When the artificial ventilation of a curarized animal ceases, there is frequently a sudden rise in blood pressure amounting to 20 mm. Hg as Traube (1865) observed. In spite of the rise in pressure the muscular blood flow decreased (fig. 2). Although the changes in blood pressure often passively altered flow in the tissues, and particularly in the brain, the typical changes in blood flow followed regardless of whether the arterial pressure was decreased by 50 mm., or increased by 20 mm. or remained constant. Apparently changes in arterial blood pressure did not initiate the differential vascular change either directly or indirectly.

Changes in breathing would have a larger influence upon the low pressure system which is involved in the venous return of blood. The return of blood from the hind legs and brain traverses quite different areas and the return through the veins anterior to the heart might be facilitated while return through the abdominal veins might be impeded. However, the vascular change in the muscles lateral to the cervical vertebrae (figs. 2, 3) corresponded with the changes in the hind leg in spite of their different venous channels. Curarization and consequent loss of venous pumping by changing muscular tone did not alter the response. Furthermore, denervation of one leg abolished the reduction in flow in it alone, but did not affect the vascular response in the other leg or neck (figs. 1, 2, 3). The effect of curare also allays the suspicion that changes in the tonic heat production of the muscles might have affected the flow recorder.

The various changes in pressure showed their mechanical influence upon the peripheral circulation, but the changes associated with arrest of ventilation were superimposed upon pressure effects. There was no common arterial or venous pressure effect which appeared able directly or indirectly to initiate the respiratory changes in the circulation.

The nervous control of the decrease in muscular flow. After the sciatic nerve was cut, changes in muscular flow occurred in response to changes in blood pressure, but the typical reduction in flow did not follow the arrest of breathing by inflation (fig. 1) or by any other method.

The principal efferent path lay in the sympathetic outflow, for after excising the first sacral and last four lumbar sympathetic ganglia, the reduction in muscular flow failed to occur in the muscles of the hind leg (figs. 2, 3). Failure of the response was limited to the denervated area and unilateral denervation did not affect the response in the other side or in the muscles of the neck (figs. 1, 2, 3).

The first two sacral and last four lumbar dorsal spinal roots were severed

in one cat, and the characteristic reduction in flow still appeared in the muscles of both legs. The persistence of the response after deafferentation was made more definite by the fact that it disappeared as usual after cutting the sciatic nerve. Evidently the dorsal root vasodilators are not essential to the response, and although it was not considered to be likely that the afferents passed through the nerve fibers from the muscles, they were shown not to contain the essential sensory path.

On account of its known influence upon cardiac activity during inflation of the lungs, the vagus nerve was first considered as a possible afferent path for exciting the vasoconstrictor effect. In seven animals, the effect was not observed after section of the cervical vagi. But in the recently vagotomized animal, the control of respiration in general is so impaired as to leave a preparation which is inefficient in making respiratory adjustments, and the irregularity of blood pressure made the determination of whether or not the decrease in flow occurred technically difficult. Instability of the vagotomized preparation also made difficult the execution of a normal response for which the essential nerve path might still persist. Further experiments on three vagotomized animals in which blood pressure and respiration were regular showed the usual reduction in muscular blood flow when breathing was arrested.

The carotid sinus regions were denervated in order to see if they were essential for the reduction in muscular blood flow and the response still occurred (fig. 4). The absence of the sinus receptors usually accentuated the reduction in blood flow and delayed recovery of normal flow after breathing was resumed. Severing the depressor nerves did not destroy the muscular flow response, nor did the combined effect of cutting the vagus and depressor nerves and denervating the carotid sinus regions. The variety of conditions which we have used to start the reduction of the muscular blood flow is so great that no single stimulating condition is suggested except the cessation of breathing and the onset of asphyxia.

Control of the cerebral blood flow. The obscurity of the cerebral circulation led to the denial of any part of vasomotor control in its regulation, but Forbes and his associates and Schmidt have shown instances of nerve control (reviewed by Wolff, 1936). Vasoconstrictors pass to the brain in the cervical sympathetics and vasodilators in the seventh nerve. The cerebral circulation appears to be less actively controlled by its known nervous connections than the muscular circulation, but we have observed the following indications of nervous regulation of blood flow in the brain.

Electrical stimulation of the cervical sympathetics produced a small but definite decrease in cerebral flow. Cutting the cervical sympathetics did not remove the increase in cerebral flow which followed the arrest of ventilation by any method. Usually the increase in flow was greater after sympathectomy than before. This appears in the comparison of the flow

changes in the normal and sympathectomized brain (figs. 5, 6). During this period the arterial blood pressure was on the whole lower in the sympathectomized animal. The accentuation of flow changes after sympathectomy had appeared when no appreciable change in arterial pressure was observed, and so it is not attributable to differences in arterial pressure. After ventilation was resumed, the recovery rise in blood pressure qualitatively was similar in the normal and sympathectomized animal, but the cerebral flow increased much more in the sympathectomized brain. This sort of observation which was quite regular after sympathectomy, resembles the observation of the abnormally rapid and large changes in blood flow which were produced passively by increases in arterial pressure in the sympathectomized muscles.

Section of the vagus and depressor nerves did not abolish the increase in cerebral flow in any preparation. Although vagotomy apparently disturbed the control of muscular flow more than it affected the cerebral flow, the difference is probably in the relatively protected situation of the cerebral circulation rather than in an essential difference in the nature of the systems controlling cerebral and muscular vessels.

After denervation of the carotid sinus regions the increase in cerebral flow was greater than it was before denervation (fig. 4). In the example given the arterial pressure rose more than it did before denervation, and this observation was frequent and to be expected.

The relation of CO_2 or lack of O_2 to the response. The inhalation of CO_2, or less noticeably, lack of oxygen, also reduces blood flow through the muscles and increases flow through the brain (Lennox and Gibbs, 1932; Irving and Welch, 1936). The arrest of alveolar ventilation in our experiments would also develop the conditions of increased CO_2 and deficient oxygen supply. A further likeness to the CO_2 effects lies in the fact that the effect of CO_2 upon muscular flow is abolished by denervation (Keller Loeser, Rein, 1930). But the vascular change after cessation of ventilation occurred within 15 seconds, and Keller, Loeser, and Rein (1930) reported that the reduction in muscular flow appeared about 30 seconds after 10 per cent CO_2 was inhaled. For the purpose of comparison I have observed the effect of the inhalation of 10 per cent CO_2 mixtures and of mixtures deficient in O_2, and the most rapid appearance of the vascular changes was 20 seconds after the start of inhalation. It seems unlikely that the pressure of CO_2 would be raised so rapidly by the cessation of breathing as by the inhalation of 10 per cent CO_2. The inhalation of 10 per cent CO_2 also increased the cerebral circulation more gradually than the arrest of ventilation, and frequently CO_2 caused either no reduction in muscular flow or had only a very slight effect. In respect to velocity and intensity the effect of CO_2 was unlike the effect of cessation of ventilation.

Discussion. When breathing ceases there are changes in the control

of the muscular operations of ventilation of the lungs, in cardiac action and in the vascular system. The control of ventilation and cardiac action is nervous and to a large degree is effected by reflex mechanisms. The method of control of the vascular response to the cessation of breathing is not obvious, but the sympathetic nerve supply provides the essential efferent pathway for reducing the flow in the muscles. The cervical sympathetic supply was not essential for increasing the cerebral blood flow. However, the cerebral vasodilator fibers in the seventh nerve (Chorobski and Penfield, 1932; Cobb and Finesinger, 1932), which would be more likely to cause increased cerebral flow, were not cut.

The search for a sensory path effective in the vascular response to the cessation of breathing was not productive, for cutting the vagus, depressor and carotid sinus nerves did not destroy the response. There are, however, a number of local and general vascular adjustments which do not involve these sensory nerves, and our knowledge of receptor systems for vascular control is poor in comparison with the control of other systems. In view of the fact that our knowledge of the sensory paths of vascular reflex adjustments is so scant the failure to demonstrate a path in the vagus, depressor and carotid sinus nerves does not discredit the possibility of reflex control.

The condition which initiates the vascular adjustment during the cessation of breathing is not definable as a physiological stimulus unless one infers that the accumulation of CO_2 is the basic condition. The effect of CO_2 differs in a temporal and quantitative fashion from the effect of the cessation of breathing, but those differences are not decisive. There seems to be no advantage from a physiological point of view in assuming that CO_2 is the stimulating agent because the site and mode of action of CO_2 in effecting vascular and even general respiratory adjustments is still obscure.

Although the details of control of the vascular adjustment during the cessation of breathing are not known, the adjustment of the blood flow in the tissue nevertheless occurs in a prompt, regular and apparently purpose manner. It may very well be regarded as an important factor in survival during asphyxia and it provides an interesting example of the correlation of the blood flow in the tissues with the other functions which are concerned in respiration.

SUMMARY

When ventilation of the lungs ceases, the flow of blood through the muscles diminishes while flow through the brain increases. The vascular responses do not depend upon the presence or absence of breathing movements.

The arterial blood pressure may fall, remain constant, or rise with different methods and conditions of arresting the ventilation, but the

vascular response is the same. Intrathoracic pressure and pumping are so differently affected in the various experiments that the condition of the venous return is not a determining factor.

The efferent path controlling the muscular response lies in the sympathetics. The carotid sinus and vagus-depressor systems are not the essential afferents.

For the cerebral vascular change the cervical sympathetics, carotid sinus regions, and vagus-depressor nerves are not necessary.

Carbon dioxide has the same effect upon the circulation but it is slower and less effective than the arrest of breathing.

The effect of arrest of ventilation upon the vascular system appears to afford a protective adjustment against asphyxia which could only be correlated by nervous control.

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RECOVERY FROM MUSCULAR ACTIVITY AND ITS BEARING ON THE CHEMISTRY OF CONTRACTION

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It has generally been accepted that the reactions which furnish the energy for muscular contraction are anaerobic, and that oxygen is required only for the processes of recovery. This was the result of the interpretation which Hill (1920) placed upon his observation that the heat liberated in the contraction itself is the same in amount, relative to the tension developed, whether oxygen is present or absent from the system.

Three separate anaerobic reaction systems have been postulated as the source of the energy for contraction. The first of these, proposed by Meyerhof (1920), was that the breakdown of glycogen to lactic acid furnished the energy. The second, by Lundsgaard (1930), based on the findings in iodoacetate-poisoned muscle contracting anaerobically, held that the hydrolysis of phosphocreatine was the reaction which supplied the energy, and that the formation of lactic acid served to resynthesize this substance and was therefore a recovery process. The third, by Lohmann (1934), regards the hydrolysis of adenosine triphosphate as the immediate source of energy for contraction, and holds that the resynthesis of this compound by transfer of phosphate groups from phosphocreatine constitutes the first stage of recovery.

The theories of Meyerhof and Lundsgaard were derived from the results of experimentation with isolated frog muscles; that of Lohmann resulted from a study of the enzyme reactions which could be made to take place in cell-free muscle extract. In 1933 the writer began to investigate the reactions taking place in contracting mammalian muscle with intact circulation and innervation. The findings (Sacks and Sacks, 1933) could not be interpreted satisfactorily on the basis of either the Meyerhof theory or that of Lundsgaard; hence an alternative theory was proposed. The essence of this theory is that the fundamental reactions in contracting muscle are oxidative and not anaerobic, and that anaerobic reactions take place only when the oxygen supply is inadequate to support a fully aerobic metabolism. The formation of lactic acid is considered to be the principal anaerobic reaction; the formation of hexosemonophosphate from glycogen and phosphocreatine also supplies energy when the oxygen sup-

ply is inadequate. The hydrolysis of phosphocreatine, on the other hand, serves the function postulated for it by Fiske and Subbarow (1929): it supplies alkali to neutralize the lactic acid formed during oxygen deficiency.

The steady state is, by definition, the condition in which the supply of oxygen to the contracting muscles is great enough so that the accumulation of anaerobic metabolites is prevented. If it is assumed that the anaerobic reactions do take place in contraction even in the presence of oxygen, and that oxygen is required only for recovery, then the rate at which the recovery process itself takes place must be sufficient to account for the non-appearance of anaerobic metabolites during the steady state. Any postulated anaerobic reaction from which the oxidative recovery is significantly slower than required for the maintenance of the steady state, becomes suspect. It has already been shown (1935b) that the lactic acid cycle does not meet these requirements, since the muscle with intact circulation does not convert lactic acid to glycogen. It has also been shown (1935a) that the phosphocreatine cycle is too slow to meet the requirements of steady state tetanus.

In the experimental work here presented it is shown that the phosphocreatine cycle is too slow to meet the requirements of a steady state of repeated single twitches involving a smaller energy output per unit time than does tetanus. It is also shown that the adenosine triphosphate cycle is very much slower than the phosphocreatine cycle, and that the resynthesis of adenosine triphosphate is not accomplished by phosphate transfer from phosphocreatine, as postulated by Lohmann. The procedure was essentially that used in the previous studies. One gastrocnemius muscle of a cat was stimulated, the other left in the resting state. Stimulation was at the rate of two shocks per second through the nerve, by means of condenser discharges through a thyratron tube into the primary of an inductorium. This rate of stimulation was found necessary (1937) to obtain any appreciable amount of adenosine triphosphate hydrolysis. The stimulation period was five minutes in every case, and recovery periods of five, ten, and twenty minutes were used. In one series of experiments the circulation was intact throughout; in the other series, the circulation was diminished, but not completely cut off, for the duration of the stimulation period only, by means of a bulldog clamp placed on the popliteal artery and vein. The tendon was cut away from the isometric lever immediately after the stimulation ended, so that the entire recovery period was passed with the muscle in the relaxed state. Both muscles were frozen with a mixture of carbon dioxide and ether at the end of the stimulation or recovery period. Analyses for the various phosphorus fractions were made by the methods used previously (1933). Ammonia was determined by the method of Williams and Nash (1933).

The muscles with intact circulation showed steady state tensions of $\frac{1}{3}$ to $\frac{2}{3}$ of the maximal tension developed. In the diminished circulation experiments, the muscles were almost completely fatigued by the end of the stimulation period. The amounts of adenosine triphosphate and phosphocreatine hydrolyzed were not significantly different in the two series.

The results are shown in tables 1 and 2. If the over-all changes in recovery are considered, the findings are essentially the same in the two series. The apparent breakdown of adenosine triphosphate in the first five minutes of recovery in the steady state series is probably experimental variation. It will be noted that the resynthesis of phosphocreatine is much more rapid than that of adenosine triphosphate, and that the two reactions proceed together. Ammonia formation, from the conversion of adenylic acid to inosinic, is less prominent in the diminished circulation experiments than with full oxygenation.

If phosphocreatine hydrolysis were the source of energy for contraction, as postulated by Lundsgaard, then it would require the hydrolysis of all this substance which was formed during five minutes of recovery to supply enough energy to maintain the level of steady state activity here present for only half a minute. In other words, the situation is such that the anaerobic theory is required to assume ten times as rapid a synthesis of phosphocreatine between successive twitches as actually takes place after the last twitch. It is not oxygen deficiency which limits the resynthesis of phosphocreatine in recovery, for Millikan (1937) has shown that the oxygen tension within the muscle rises within a few seconds of the cessation of tetanus, to the level present in resting muscle. The factor limiting the rate of phosphocreatine resynthesis has been shown (1935a) to be the rate of removal of lactic acid from the muscle; this is primarily a non-oxidative process, as it is carried on principally by diffusion into the blood stream.

The corresponding experiment has been performed on isolated frog muscle under conditions in which the oxygen supply was constant throughout and was sufficient to permit a much more rapid resynthesis of phosphocreatine than actually did take place. Part of the work was done by Mawson (1932, 1933), part by Grimlund (1936), and part by Cori, Cori, and Hegnauer (1937). Mawson found that iodoacetate treated frog sartorii supplied with lactate and oxygen could give ten twitches a minute without decomposing any significant amount of phosphocreatine. Grimlund used bromacetic acid to inhibit lactic acid formation, and a stimulation rate of 14 to 16 per minute. He found no significant decrease in tension after over an hour of activity under these conditions. Cori, Cori, and Hegnauer determined the rate of disappearance of hexosemonophosphate in iodoacetate frog muscles in oxygen. This is the equivalent of determining the rate of recovery in the experiments of Mawson and Grimlund, for it is hexosemonophosphate which is formed when the poisoned

muscles give single twitches anaerobically. Lundsgaard's (1930b) data show that 0.3 mgm. per cent of hexosemonophosphate-P is formed per

TABLE 1

Resynthesis of phosphocreatine and adenosine triphosphate in recovery from contraction with circulation intact

Values are expressed as milligrams per cent of P and N

PHOSPHOCREATINE HYDROLYZED	ADENOSINE TRIPHOSPHATE HYDROLYZED			RESIDUAL PHOSPHOCREATINE
	P	N	N calculated*	
a. Stimulation 5 minutes, no recovery				
45	12	2.8	2.7	12
47	9	1.9	2.0	15
34	5	0.8	1.1	15
44	9	1.9	2.0	16
54	5	1.9	1.1	15
56	9	2.5	2.0	12
Av. 47	8	2.0	1.8	14
b. Stimulation 5 minutes, recovery 5 minutes				
29	12	3.2	2.7	
20	8	2.1	1.8	
26	13	2.4	2.9	
20	8	1.6	1.8	
17	10	1.8	2.3	
Av. 22	10	2.2	2.3	
Phosphocreatine resynthesized				25
Adenosine triphosphate resynthesized				(−2)
c. Stimulation 5 minutes, recovery 10 minutes				
10	11	1.9	2.5	
0	2	0.4	0.4	
13	2	1.6	0.4	
−6	6	1.0	1.3	
7	4	1.2	0.9	
Av. 5	5	1.2	1.1	
Phosphocreatine resynthesized (entire recovery)				42
Adenosine triphosphate resynthesized (entire recovery)				3

* From phosphorus value.

twitch under these conditions. This would amount to 3 mgm. per cent per minute in Mawson's experiments and 5 mgm. per cent per minute in

TABLE 2

Resynthesis of phosphocreatine and adenosine triphosphate in recovery from contraction. Circulation diminished during contraction, normal in recovery

Values are expressed as milligrams per cent of P and N

PHOSPHOCREATINE HYDROLYZED	ADENOSINE TRIPHOSPHATE HYDROLYZED			RESIDUAL PHOSPHOCREATINE
	P	N	N calculated*	
a. Stimulation 5 minutes, no recovery				
48	13	2.1	2.9	5
43	14	2.7	3.2	12
49	11	1.8	2.5	10
47	10	1.9	2.3	9
51	8	1.4	1.8	17
Av. 48	11	2.0	2.5	11
b. Stimulation 5 minutes, recovery 5 minutes				
22	15	3.0	3.4	
29	10	2.5	2.3	
7	7	0.9	1.6	
17	4	2.1	0.9	
16	10	1.0	2.3	
Av. 18	9	1.9	2.1	
Phosphocreatine resynthesized				30
Adenosine triphosphate resynthesized				2
c. Stimulation 5 minutes, recovery 10 minutes				
21	7	2.7	1.6	
8	6	2.2	1.4	
36	6	2.0	1.4	
8	13	2.5	3.0	
2	5	1.8	1.1	
Av. 15	7	2.2	1.7	
Phosphocreatine resynthesized (entire recovery)				33
Adenosine triphosphate resynthesized (entire recovery)				4
d. Stimulation 5 minutes, recovery 20 minutes				
6	2	0.6	0.5	
1	2	0	0.5	
0	4	1.3	0.9	
Av. 2	3	0.6	0.6	
Phosphocreatine resynthesized (entire recovery)				45
Adenosine triphosphate resynthesized (entire recovery)				8

* From phosphorus value.

those of Grimlund. These are the quantities which must be resynthesized to phosphocreatine per minute, on the basis of the anaerobic theory, to explain the experimental observations. But the maximum rate of this change found was only 8 mgm. per cent per *hour*. The supply of oxygen diffusing into a frog sartorius at 15°C., the temperature used in these experiments, is great enough to permit a steady state of 37 twitches per minute, according to Hill and Kupalov (1929). There is no anaerobic resynthesis of phosphocreatine in these experiments, for Lundsgaard (1934) has shown that this does not take place in iodoacetate muscles. Furthermore, the recovery process was not impaired by the iodoacetate treatment, for the rate of hexosephosphate removal was the same in normal muscles as in the poisoned ones. The rate of lactic acid oxidation was also the same in normal and poisoned muscles. These data on frog muscle supplement those obtained on mammalian muscle in showing that the anaerobic theory must assume that the muscle is able to accomplish recovery changes much more rapidly in the interval between twitches than in an equal time interval after the last twitch, even though the oxygen tension is the same. The oxidative theory, on the other hand, is not faced with any such difficulty. In the presence of adequate oxygen, the anaerobic reactions simply do not take place.

With regard to adenosine triphosphate, the possibility that its hydrolysis can serve as the immediate source of the energy for contraction is even more remote. The total quantity of this substance resynthesized in ten minutes of recovery under full oxygenation is not much more than enough to supply energy for half a dozen twitches. The slow resynthesis is not due to lack of phosphocreatine, for even at the beginning of recovery there is a fairly large amount of this substance present. The data show rather that both substances are resynthesized together, though the rate of adenosine triphosphate resynthesis is much slower than that of phosphocreatine. According to the Lohmann theory, the resynthesis of adenosine triphosphate is rapidly accomplished by phosphate transfer from phosphocreatine. While Lohmann's findings may be applicable to the situation in cell-free extract, it is quite evident that they do not apply to the situation in intact muscle.

The findings on ammonia formation and removal enable a verdict to be given in the old argument between Embden and Parnas. Embden (1931) maintained that the ammonia liberated from adenylic acid in contraction was rapidly replaced in its original position, while Parnas (1930) stated that ammonia once formed could not be used again by the muscle. The present data indicate an intermediate position. The ammonia is only slowly replaced in the adenylic acid, but it is this ammonia, and not that furnished by the oxidative de-amination of amino acids, which is utilized. It also appears that the presence of oxygen is necessary to insure complete

removal of ammonia from the adenylic acid in the first instance, for only in the experiments with intact circulation is the ammonia formed equivalent to that calculated from the phosphate liberated, i.e., to the adenylic acid formed. In the restricted circulation experiments, only part of the adenylic acid is de-aminated to inosinic.

These findings also indicate how limited and selective the permeability of the muscle fiber is. Neither in the stimulation period nor in the fairly prolonged recovery period does any appreciable amount of ammonia escape from the muscle by diffusion.

The findings on intact muscle here presented, together with data from other sources, demonstrate that none of the three cycles of anaerobic contraction-oxidative recovery reactions that have been postulated, is capable of explaining the situation of the steady state. Only the direct oxidative theory will satisfactorily account for the observations on intact muscle. The chemical method is not able, on account of the unavoidable time lags, to offer direct proof of the oxidative nature of the fundamental reactions, for it is only anaerobic metabolites which can be measured directly. But physical methods can throw additional light on the situation and avoid the time lags to a very high degree. The shorter the instrument lag has become, as the methods have improved, the less the delay that is found between the onset of contraction and increased utilization of oxygen. Measurements on exercising man are of necessity subject to a considerable time lag. But Millikan (1937) has been able to measure the time required for an increase in oxygen utilization within the muscle to become apparent. By the use of a differential photocell colorimeter, he found that the de-oxygenation of muscle hemoglobin began within 0.2 second of the onset of contraction (tetanus). The lag is that of the recording instrument, not of the process in the muscle.

These findings make it unnecessary to postulate that anaerobic reactions furnish the energy for contraction in the presence of adequate oxygen. The data obtained by physical methods do not exclude this possibility, as Millikan is careful to point out, but they do demonstrate that any postulated anaerobic reaction must be capable of very rapid oxidative reversal; none of the anaerobic reactions which have been observed in muscle can meet this requirement. Naturally, the muscle must depend on anaerobic reactions to meet sudden emergencies. In passing from rest to vigorous activity the metabolism of the muscle may increase instantaneously to over twenty times the resting value, but the blood flow and oxygen supply require more than a minute to reach the maximum (Kramer, 1937). During this period of circulatory adjustment the work must perforce be largely anaerobic. But this does not imply that the muscle uses anaerobic reactions when the oxygen supply is adequate.

The chief stumbling block to the acceptance of the direct oxidative

theory has been the interpretation placed upon the lack of dependence of the ratio between heat and tension on the presence of oxygen. Hill interpreted the constancy of this ratio as proof that the chemical reactions taking place were the same in the presence of oxygen as in its absence. Careful analysis shows that this interpretation is not necessarily correct. The heat liberated in a series of chemical reactions is independent of the path, and depends only on the initial and final states. The constancy of initial heat in contracting muscle means only that the efficiency of converting chemical energy into mechanical work is independent of the reaction which furnishes the energy, for it is only this mechanical efficiency that the ratio of tension to heat measures.

The post-stimulation heat is a different matter. This does vary over wide limits; it is small under anaerobic conditions and large in the presence of oxygen. It is greater in mammalian muscle in oxygen after tetanus than after twitches (Cattell and Shorr, 1932). If the attempt is made to correlate the post-stimulation heat with oxidative recovery from anaerobic contraction, considerable difficulty is encountered. If there is such an oxidative restitution, and its thermal efficiency is close to 100 per cent, as has been suggested, then the heat liberated during oxidative recovery should be zero, as a balance between the endothermic resynthesis (of phosphocreatine or adenosine triphosphate) and the exothermic oxidation reactions. But the post-stimulation heat of frog muscle in oxygen is equal to the initial heat, and in mammalian muscle after tetanus it is three to four times as great as the initial heat. If this did represent a true recovery, then the efficiency of the process could not be more than 50 per cent in frog muscle, and not more than 20 to 25 per cent for mammalian muscle after tetanus. A more reasonable interpretation is that the post-stimulation heat merely represents the inertia of the tissue in returning to the resting metabolic level after a burst of activity.

Neither the anaerobic theory nor the direct oxidative theory is capable of explaining the process by means of which the muscle converts the chemical energy into mechanical work. That is an entirely separate problem, and one which has not yet been solved.

SUMMARY AND CONCLUSIONS

1. In recovery from muscular contraction, phosphocreatine is resynthesized slowly and adenosine triphosphate even more slowly. The two processes are independent of each other.

2. The ammonia liberated by the de-amination of adenylic acid in contraction remains in the muscle and is utilized for the resynthesis of the compound in recovery.

3. None of the anaerobic reactions which have been postulated to

furnish the energy for contraction seems capable of serving that function under steady state conditions.

4. The evidence showing that contracting muscle uses oxidative reactions directly as the source of energy is discussed, and it is concluded that only on this basis can the findings of the steady state be accounted for.

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THE RELATIONSHIP BETWEEN TISSUE CHLORIDE AND PLASMA CHLORIDE

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Tissues vary greatly from one another, and from the blood, in their content of chloride (see Irving and Manery, 1936, for review). All vertebrate skeletal muscle, for instance, shows low values. Tendon, on the other hand, and connective tissues in general show very high values, even exceeding that found in the red blood corpuscles. Between these two extremes the other tissues of the body fall, in an apparently haphazard manner.

The special situation in skeletal muscle has led a number of investigators to conclude that the chloride of this tissue, and probably most of its sodium as well, is not homogeneously distributed, but occurs mainly, if not exclusively, in the extracellular fluid, in equilibrium with the blood plasma. This view was developed long ago by Overton (1902). It has recently been advocated by Ernst and Takacs (1931) and Mond and Netter (1932) who have shown that amphibian muscles, placed in, or perfused with, isotonic sucrose solutions, rapidly lose their chloride and sodium while retaining practically all of their potassium and phosphate. Fenn, Cobb and Marsh (1934) and Eggleton, Eggleton and Hamilton (1937) have soaked frog muscles in a series of solutions of varying chloride content, made up by replacing various fractions of the chloride by nitrate. They find that the tissue chloride varies in direct proportion to the chloride content of the external fluid.

Harrison, Darrow and Yannet (1936) have extended the conception to all tissues of the mammalian body. Analyses of the electrolytes of the whole animal are considered by them to fit in plausibly with the assumption that all chloride, with the exception of that found in the erythrocytes, is extracellular, and may be used as a measure of the volume of extracellular water. The same view has been accepted by Peters (1935), Fenn (1936), Hastings and Eichelberger (1937), and Bourdillon (1937). On the basis of their mammalian studies Harrison, Darrow and Yannet arrive at the conclusion that extracellular water constitutes between 24 and 30 per cent

of the body weight. Their average (rabbits, dogs and monkeys) is 27 per cent.

These values are higher than those indicated by other methods. By the thiocyanate method Lavietes, Bourdillon and Klinghoffer (1936) arrive at values in normal men ranging from 20 to 28 per cent. Their average is 22.6 per cent. Sucrose gives them even lower values, ranging from 13 to 22 per cent, with an average of 18.6. The more recent sucrose measurements of Keith and Power (1937) lie between 17 and 21 per cent. The sulfate method, used by Lavietes, Bourdillon and Klinghoffer (1936) and by Bourdillon and Lavietes (1936) gives rather variable results ranging between 14 and 29 per cent, with an average of 20 per cent. Although the measurements have not been made on the same mammalian species, there is a sufficient discrepancy between these various values and that calculated on the assumption that all chloride is extracellular to raise doubt concerning the validity of the hypothesis for all tissues of the body, however cogent may be the arguments in its favor for skeletal muscle.

In studying the literature of this field it occurred to us that it would be very useful to know whether, in the intact animal, the chloride of mammalian tissues can be diminished when the plasma chloride is lowered and to establish the relationship between the two over a wide range of plasma chloride values. Certain avenues are open to permit the induction of extensive changes in plasma chloride. Pyloric obstruction reduces its value very considerably, but the accompanying dehydration is so extreme as to render the procedure inadmissible for our purpose. Plasma chloride may also be somewhat reduced by diuretic agents. It may be partly removed by the method of intestinal lavage.

None of these methods, however, gave promise of the very great reductions in plasma chloride which we considered necessary if we were to establish a general relationship over a wide range of chloride values. We turned, therefore, to another mode of approach, namely, the substitution of other anions for the chloride. Such work was first done by Nencki and Schoumow-Simanowsky (1894) who showed that the bromide ion, administered through the diet, is able to replace considerable amounts of chloride, in both blood and tissues, and even appears in the gastric juice as hydrobromic acid. An extensive literature has grown up concerning the use of bromide in such substitution work.

In opposition to the conclusions of earlier investigators, Weir (1936) who reviews the literature, finds that, after the oral administration of bromide to dogs, the blood and tissues come quickly into a definite state of equilibrium with regard to bromide and chloride. The ratio $\frac{Br}{Cl + Br}$ is identical for blood and for all other tissues studied, except the central nervous system, where the value is considerably lower. Bromide penetrates with

difficulty into the brain, a fact which may be related to its well-known inability to pass freely from blood to cerebrospinal fluid.

According to this literature the bromide ion, in massive doses, induces a severe intoxication, with muscular weakness, lack of nervous coördination, and sleepiness as some of its more obvious symptoms. The iodide ion is also very toxic. In both cases death ensues before any considerable fraction of the chloride has been displaced. The chemical separation of these two halides from chloride, in tissue samples at least, is a difficult technical feat. For several reasons, therefore, they are not admissible for our purpose.

METHODS. We have turned, therefore, to anions not belonging to the halide group. We have found that in cats, used throughout the present study, sulfate furnishes a very acceptable substitute for chloride. It is normally present in the blood and may be greatly increased without obvious physiological reaction, at least in such acute experiments as furnish the data for the present report. The blood volume and blood pressure are well maintained for several hours as the substitution proceeds. The animals may recover consciousness and exhibit essentially normal behavior for some hours, even after the replacement of more than half of the body chloride.

We have substituted sulfate for chloride in three ways, as follows:

1. *By total plasmapheresis.* The technic is identical with that described by Stanbury, Warweg and Amberson (1936). Amberson (1937) gives a fuller discussion of the literature.

The artificial serum consists of:

Na_2SO_4	14.60 grams	Glucose	1.00 gram
K_2SO_4	0.33 gram	Gum acacia	60.00 grams
$CaSO_4$	0.30 gram	Water to	1000.00 cc.
$NaHCO_3$	0.40 gram		

To make each liter of this fluid we use two 100 cc. ampoules of Lilly's acacia without sodium chloride.

In this serum are suspended washed ox (or cat) red cells, previously rendered chloride free. While some investigators (Woodhouse and Pickworth, 1932; Bourdillon and Lavietes, 1936) have claimed that sulfate ion cannot penetrate into the erythrocyte, others have reported a fairly ready penetration (Mond and Gertz, 1929). It has long been known that red cells shrink in volume when washed through sulfate solutions made up to contain the same concentration of ions as isotonic Ringer-Locke solution. The loss in volume occurs because divalent sulfate exchanges for two chloride ions, the osmotic pressure of the cell interior is reduced, and water is lost to the exterior.

We find it possible to remove all but the last traces of chloride from ox and cat cells when they are repeatedly washed through colloid-free sulfate-

Ringer-Locke solutions. After seven centrifugings and resuspensions, the chloride is reduced to less than 5 mgm. per cent. The cells withstand this treatment with little hemolysis, and their ability to transport oxygen is not impaired. In the final perfusion mixture there must be 30 to 40 per cent of such cells.

In our best experiments we have been able to pass very large quantities of this fluid through the blood vessels of the cats, infusing through a jugular cannula and simultaneously bleeding from the carotid. In several cases the volume of fluid passed has exceeded twenty times the normal blood volume, and has actually surpassed the volume of the cat itself. The gum acacia protects the animal from edema, so that the tissues, at the end, are normal in color and size, with a normal water content.

Most of our data have been secured by this method. We have, however, employed two other supplementary methods in a few experiments.

2. *By diuresis.* Colloid- and cell-free sulfate-Ringer is very slowly infused into the cat's body through a jugular cannula (120 to 150 cc. per hour). Diuresis soon begins, and great quantities of urine are collected as the experiment proceeds. Some chloride is washed out of the body in the urine. As the infusion proceeds, however, the concentration of chloride in the urine falls, until finally it is practically chloride free. It is not possible, therefore, to reduce the plasma chloride by more than 25 per cent by this method.

3. *By intestinal lavage.* Previous investigators have employed distilled water, or isotonic sugar solutions, for intraperitoneal injection. We have used instead sulfate-Ringer-Locke without cells or colloid. Without anesthesia 300 cc. of this solution are injected and allowed to remain within the peritoneum for two or three hours. As much as possible of the fluid is then withdrawn. The fluid now contains a great number of leucocytes and a considerable amount of plasma protein. It also contains chloride in considerable quantities, so that, by successive injections and withdrawals, a large fraction of the body chloride can be washed away. Unfortunately the animals take the operation badly. They soon show evidences of muscular weakness and shortly pass into a coma in which all of the skeletal muscles show fibrillary twitchings. These are of nervous origin, and disappear when a light ether anesthesia is imposed.

Chloride determinations. Filtrates of blood, corpuscles or plasma in the conventional 1:10 dilution are prepared by the zinc hydroxide procedure of Somogyi (1930). Peritoneal washings are also cleared of protein by zinc hydroxide precipitation. Fluids such as urine and aqueous humor are used directly after appropriate initial dilution.

Tissue filtrates suitable for chloride analyses are obtained by a modification of the alkaline digestion procedure suggested by Sunderman and Williams (1933). The tissues are cut into small pieces with scissors and

weighed in small Erlenmeyer flasks. Forty per cent NaOH is added in the proportion of 0.25 ml. per gram of tissue, and the mixtures are digested in the steam bath until the tissues are completely disintegrated. The alkaline digests are then transferred to volumetric flasks of such capacity that 25 ml. of diluted digest represent approximately 2 grams of tissue. After partial dilution with water, the alkaline digest is neutralized by addition of a solution of aluminum nitrate, 4 ml. of which is equivalent to 1 ml. of 40 per cent NaOH. After the mixture has cooled to room temperature water is added to volume. The mixture is thoroughly shaken, centrifuged, and decanted through chloride-free paper. Filtrates thus obtained are clear and, for most tissues, only slightly colored.

TABLE 1

Effect of perfusion upon blood and tissue chloride

	CHLORIDE				CHLORIDE		
	Average normal	After perfusion	Per cent at end		Average normal	After perfusion	Per cent at end
	mM/kg.	*mM/kg.*			*mM/kg.*	*mM/kg.*	
Blood plasma*	117	7	6	Liver	38	2	5
Blood cells*	74	6	8	Cerebrum	42	32	76
Muscle	13	2	15	Cerebellum	42	31	74
Lung	66	7	11	Spinal cord	43	27	63
Kidney	64	4	6	Sciatic nerve	62	13	21
Stomach	59	18	30	Testis	60	11	18
Spleen	48	5	10	Tendon	82	29	35
Salivary gland	50	8	16	Skin	42	12	29
Pancreas	46	9	20	Bone	27	9	33
Intestine	44	7	16	Bone marrow	33	15	46
Heart	41	3	7	Aqueous humor*	124	40	32

*mM/L.

Duplicate 25 ml. aliquots of filtrates prepared as above are taken in 50 ml. volumetric test tubes (Williams and Nash, 1933). After addition of a controlled excess of standard $AgNO_3$ solution (1 ml. = 1 mgm. NaCl) followed by 5 ml. of concentrated HNO_3 containing 6 per cent of ferric alum, the tubes are heated in boiling water to coagulate the silver chloride. The tubes are then cooled to room temperature, water is added to volume, the contents mixed, and filtered through chloride-free paper. A 25 cc. aliquot of the final filtrate is titrated with standard NH_4CNS (1 ml. = 1 ml. $AgNO_3$) from a micro-burette graduated in 0.05 ml. divisions.

Adequate blank analyses are run. The recoveries of added chloride are satisfactory.

Results. The results here reported are based upon chloride analyses of blood and tissues made on five normal animals, on twelve animals in

which chloride was reduced by plasmapheresis, on two in which the reduction was achieved by diuresis, and on two in which intestinal lavage was employed. In seven of the twelve plasmapheresis experiments we continued the perfusion until the animal died. In one the solution was exhausted shortly before death, the animal living for about thirty minutes after perfusion ceased. In four animals the perfusion was terminated at various times short of the period previously established as the lethal range. These four animals all recovered consciousness and lived for three to five hours, after which time they were killed and blood and tissue samples taken, giving chloride values in the middle range. The readings by the other two methods also fall within this range and confirm the values secured by plasmapheresis.

Protocol of a typical experiment: Plasmapheresis experiment of June 10, 1937. Male cat. Initial weight 2.47 kilos. Weight at death 2.41 kilos. Perfusion started 11:30 a.m., terminated 2:45 p.m. Average perfusion rate 13.5 cc/min. Total quantity infused into jugular 2636 cc. Total quantity withdrawn from carotid 2635 cc. No edema. Respiration and heart beat normal until 2:35 p.m. Total chloride removed from the body (by analysis of outflow fluid) about 2.4 grams. Blood and tissue samples gave chloride values shown in table 1, contrasted with the normal average. Potassium in perfused muscle 21.3 mgm. per cent; in normal control 21.4 mgm. per cent. Potassium in perfused kidney 10.5 mgm. per cent; in normal control 10.9 mgm. per cent.

All tissues lose chloride, but the percentage of reduction varies greatly from tissue to tissue. In a few tissues, such as the blood corpuscles, muscle, lung, kidney, spleen, heart and liver the terminal percentages are not far different from that found in blood plasma, indicating that these organs quickly come approximately into equilibrium with the blood. Other organs, such as cerebrum, cerebellum and spinal cord, retain most of their chloride, and considerable fractions also remain in such relatively non-vascular tissues as tendon, skin and bone. The stomach continues to hold nearly a third of its chloride.

The significance of the terminal chloride values becomes more clear when we inspect the data from all experiments. We may examine, first, the relationships observed in red blood cells and in skeletal muscle (biceps femoris). These are shown in figure 1. In both tissues the chloride changes very nearly in direct proportion to the variations in plasma chloride, although the muscle data are somewhat irregular. The best straight lines through the points pass directly through the origin, showing that in both tissues all of the chloride is diffusible.

Our most regular data have been secured in analyses of kidney and lung, plotted together in figure 2. The slopes of the lines drawn through the two sets of data are identical, but in the lung the line does not pass through the origin. There is, instead, a definite though small y-axis intercept,

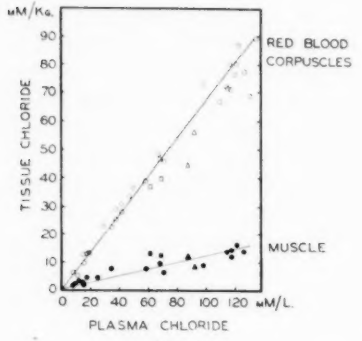

Fig. 1

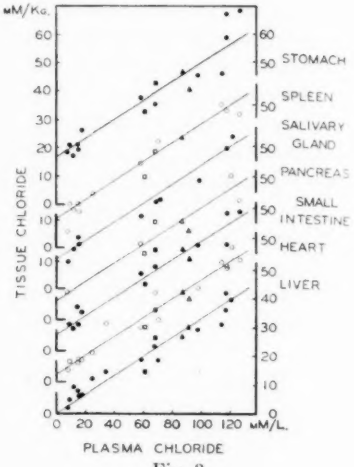

Fig. 3

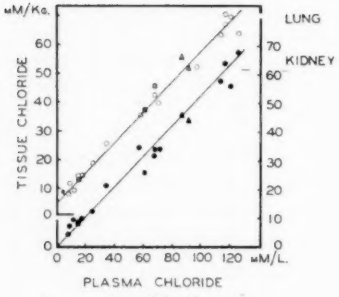

Fig. 2

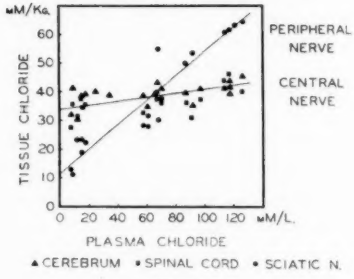

Fig. 4

Figs. 1-4

Fig. 1. Relationship between plasma chloride and chloride of the red blood cells and skeletal muscle.

Fig. 2. Relationship between plasma chloride and chloride of lung and kidney.

Fig. 3. Relationship between plasma chloride and chloride of a group of tissues in which the diffusible fraction appears to have the same concentration.

Fig. 4. Relationship between plasma chloride and chloride of nerve tissues.

In Figs 1-3 the values obtained by total plasmapheresis are shown by open or closed circles (○ ●), those by diuresis as triangles (△ ▲), and those by intestinal lavage as squares (■ □)

suggesting the presence of a small amount of indiffusible chloride. We may conclude that the major part of lung chloride and all of the kidney chloride is in equilibrium with blood plasma.

The existence of indiffusible chloride is even more strongly suggested

by the data for other tissues. In figure 3 we have brought together the results obtained for liver, heart, small intestine, pancreas, salivary gland, spleen and stomach. They are arranged in the order of the magnitude of of the y-intercept, from the liver, where the line passes through the origin, to the stomach, where the y-intercept attains the high figure of 17 mM/kg. or about 30 per cent of the total chloride of the normal tissue.

The slopes of the straight lines drawn through the experimental points are identical for all seven tissues of figure 3, indicating that in all the diffusible chloride has the same concentration, although the indiffusible fraction varies considerably. We observed this apparent identity in diffusible chloride only after all of the data had been plotted. We had not expected any such relationship, and can give no explanation for it.

Nervous tissues fall into a very different and unique category. The data for cerebrum, spinal cord and sciatic nerve are shown in figure 4. The central nervous structures resist chloride removal in a truly remarkable manner. The y-intercept of 34 mM/kg. is over 80 per cent of the normal average. At the same time that brain and cord are tenaciously holding their chloride the sciatic nerve suffers a great loss, but a y-intercept of 11 mM/kg. suggests the presence in it of a small indiffusible fraction.

Discussion. The data here presented prove that the major part of the chloride of the mammalian body is diffusible. In some tissues sulfates may replace all, or nearly all, of the chloride. The existence of an indiffusible fraction in many of the tissues is, however, strongly suggested.

This indiffusible fraction is presumably intracellular. It is held within the cells for one, or all, of the following reasons:

1. It is in part organically bound.

2. Though free, in whole or in part, it cannot readily pass through the living membranes, which, with the single exception of the erythrocyte, are believed to be relatively impermeable to anions.

3. Even a slow permeation is rendered impossible by the fact that sulfate ion is completely unable to penetrate the living cell membranes (other than the erythrocyte), so that it cannot exchange with intracellular chloride.

We incline to the opinion that the last alternative may be most important of all. Of all anions which might be employed in work of this character sulfate is probably best suited for the demonstration of intracellular chloride. Its inability to penetrate such membranes as the gut wall and kidney tubule is well known. It apparently exchanges readily enough with chloride in extracellular water (Lavietes, Bourdillon and Klinghoffer, 1936; Bourdillon and Lavietes, 1936), but probably finds more difficulty than any other anion in crossing the living cell membrane. It cannot, therefore, replace intracellular chloride, whose presence is thus made manifest in all tissues where it occurs.

This conclusion is, we believe, supported by a comparison of our data with that of Weir (1936). He found no evidence that any of the tissues have indiffusible chloride, except in the central nervous system. In one of his experiments (dog) bromide replaced 33.4 per cent of plasma chloride, 32.0 per cent of lung chloride and 32.8 per cent of bone chloride. In the cerebrum, however, bromide replaced only 19.5 per cent of the chloride. At equilibrium the plasma chloride was 73.2 mM/kg. At this level of plasma chloride in our experiments sulfate is able to replace only about 7 per cent of cerebral chloride. We may conclude, therefore, that some bromide is probably able to penetrate into the cerebral cells, and that an equivalent amount of chloride is able to diffuse out, whereas sulfate does not go in at all, so that no intracellular chloride is lost. If sulfate replaces only extracellular chloride, then the volume of extracellular water in the cerebrum cannot be more than about 10 per cent of its total volume.

Under our experimental conditions chloride equilibrium is not obtained immediately throughout the whole body, as perfusion proceeds. Even with relatively slow perfusion there is some lag. Thus in one experiment the plasma chloride was 49 mM/L at the end of perfusion, but three hours later had risen to 68 mM/L. In another experiment where the animal lived for about half an hour after conclusion of perfusion the plasma chloride rose from 13 mM/L to 22 mM/L. Chloride is obviously being mobilized from some of the less accessible tissues, and redistributed throughout the body.

This redistribution appears to be complete in about 30 minutes. Other anions are known to reach equilibrium in about the same time (Weir, 1936). Sulfate similarly distributes itself throughout extracellular water within a few minutes after injection (Bourdillon and Lavietes, 1936).

The y-intercepts of figures 2, 3 and 4 are largely determined by groups of experimental points in the low chloride range. With the single exception of one cat which survived for half an hour these values were secured from animals which died upon the operating table while perfusion was proceeding, so that chloride equilibrium between plasma and tissues could not have been fully attained.

Yet these intercepts are so large in some tissues that it seems highly improbable that they all arise from delay in the attainment of chloride equilibrium. Certainly in the central nervous system the retention of chloride is too striking a phenomenon to permit any such interpretation. While we have not yet been able to hold animals for long in the low chloride range we have succeeded, in several cases of intestinal lavage, in keeping them alive for seven or eight hours. Here equilibrium must have been complete, yet brain and cord gave chloride values only slightly less than the normal averages although plasma chloride had for hours been about fifty per cent of normal.

Fenn, Cobb, Hegnauer and Marsh (1934) calculate that the "chloride

space" of fresh amphibian peripheral nerve must be 50 per cent of its whole volume. When various fractions of the chloride are replaced by nitrate they find that in excised nerves, as in muscle, the tissue chloride varies directly with the plasma chloride with no evidence of an indiffusible fraction. Either mammalian peripheral nerve differs from that of the lower vertebrates or our experimental conditions are dissimilar from those under which Fenn and his colleagues worked, since the cat sciatic data suggest that about one-sixth of peripheral nerve chloride is indiffusible. Possibly nitrate is able to penetrate into the cell, whereas sulfate, as previously argued, is not.

The existence of a direct proportionality between tissue and plasma chloride has been accepted by some investigators as proof that chloride is extracellular. In blood cells, however, where chloride is known to be intracellular, the same relationship exists. We must insist, therefore, that this relationship does not in itself prove anything about the location of chloride within the tissues. The theory must rest rather upon such observations as those of Hastings and Eichelberger (1937) and Eggleton, Eggleton and Hamilton (1937) who observe a great increase in the volume of the chloride space when muscle tissue is killed.

The chloride content of several tissues is very high indeed. In tendon it even exceeds that found in the red blood corpuscles. In this tissue the calculated chloride space becomes 75 per cent. Since tendon contains only about 70 per cent of water the conclusion is inescapable that some of the chloride must be intracellular. Manery (1937) has recently emphasized the high chloride content of connective tissue, and concludes that in all tissues the chloride space may be regarded as closely related to the "connective tissue phase" and as containing a considerable amount of protein. In mammalian lung tissue the calculated chloride space is about 60 per cent, the water content about 78, so that nearly 80 per cent of the water must be assigned to dissolve the chloride. In this and several other tissues the probability becomes very great that some of the chloride is intracellular. By several lines of reasoning, therefore, we are led to the conclusion that the concept of extracellular chloride, however well established for muscle, must not be extended uncritically to all other tissues.

The behavior of muscle chloride in our experiments demonstrates the normality of this tissue up to the very end of our perfusions, since there is no evidence of any increase in the chloride space such as is known to occur in injured or dead muscle. The normality of the tissues is also proven by their retention of a normal potassium content at the same time that chloride has been drastically reduced.

SUMMARY

1. Most of the chloride of the cat's body is diffusible. It may be removed by long perfusion with Ringer-Locke solution made up with the

sulfates of sodium, calcium and potassium instead of the chlorides. To this solution are added chloride-free beef cells and gum acacia.

2. By this technic plasma chloride has been reduced to as low as 6 per cent of normal.

3. In certain tissues, such as the red cells, skeletal muscle, the liver and the kidney all of the chloride is diffusible and tissue chloride varies directly with plasma chloride.

4. In other tissues such as stomach, spleen and salivary glands there is evidence for an indiffusible chloride fraction which is not removed by perfusion, in addition to a diffusible fraction which varies directly with plasma chloride.

5. This retention of chloride is particularly striking in central nervous tissue. Cerebrum, cerebellum and spinal cord all hold their chloride tenaciously, and lose very little even when plasma chloride has been greatly reduced. At the same time, peripheral nerve loses most of its chloride.

6. It is concluded that the theory which holds that chloride is able to penetrate only into extracellular water must not be extended uncritically from muscle to all other tissues. Our data suggest very strongly that in some of the tissues a considerable fraction of chloride is intracellular.

7. Except in the erythrocyte, the sulfate ion probably cannot penetrate the living cell membranes. It cannot, therefore, exchange with intracellular chloride, whose presence is thus made manifest in all tissues where it occurs.

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THE EFFECT OF THE EXTRAVASCULAR SUPPORT OF THE VENTRICLES ON THE FLOW IN THE CORONARY VESSELS

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The rate of blood flow through the coronary vessles is determined *directly* by only three simultaneous variables: 1, the driving pressure at the mouths of the coronary arteries less the pressure at the coronary portals of exit; 2, active changes in calibre of the coronary vessels due to changes in tone of the smooth muscle in their walls, and 3, passive changes in calibre of the vessels caused by the changing extravascular force exerted on intramural vessels by the surrounding cardiac musculature. The first variable is the pressure factor and the last two compose the resistance factor. Obviously in the intact animal these three directly responsible factors are not only dependent on each other but are in turn interrelated in a complex network with numerous other factors, the whole forming a sensitive regulatory mechanism. In any approach to the problem of the regulation of the coronary circulation, one must take into account this complex interrelationship of governing factors and interpret results accordingly.

In determining the factors concerned in regulating coronary blood flow, one is faced with the choice of two methods of approach. In the first method the coronary flow is measured in the intact unanesthetized (Essex, Herrick, Baldes, and Mann, 8, 10) or anesthetized animal (Rein, 14) with conditions as nearly normal as it is possible to make them. The flows so obtained depict the coronary flow as a resultant of all the controlling factors involved, but the evaluation of each individual factor is a matter of deductive reasoning. In the analytical method such as Anrep et al. (1, 2) and Wiggers (18, 19) have used, the coronary flow is measured in an abnormal but carefully controlled preparation, so that the effect of each individual factor alone may be determined. But the part each plays in the intact animal is a matter of inductive reasoning, since with this

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method only what *can* happen is determined and not what *does* happen in a normal animal. It is only by a combination of the results from these two methods of approach that the entire picture of coronary flow can be completed.

It was our purpose to evaluate one of the directly controlling factors, viz., the passive changes in calibre of the coronary vessels due to the changing extravascular force exerted by the heart muscle under different conditions of contraction. This we attempted to approach on an anlytical basis. For this purpose, we have developed an assembly, after considerable preliminary trial, in which all other factors could be kept constant and the desired variable controlled at will.

METHOD. Dogs averaging 15 to 20 kilos were anesthetized with sodium barbital, the chest opened, and artificial insufflation of the lungs instituted.

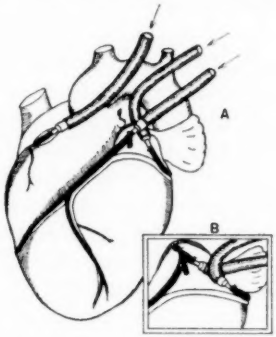

Fig. 1. A, diagram showing method of inserting coronary cannulae in all experiments except no. 61. Insert B, method of cannulation in experiment 61.

The blood vessels to the head and body were tied off and the preparation completely denervated. The blood was rendered noncoagulable by injecting heparin intravenously (7 mgm/kilo., Connaught Laboratories preparation). A heart-lung preparation was thus formed with the usual artificial peripheral resistance connected to the aorta and a venous reservoir containing defibrinated dog's blood connected to the superior vena cava. In a number of instances the lungs were also removed and replaced by an artificial peripheral resistance and venous reservoir connected respectively to the pulmonary artery and left auricle. The latter procedure gave a completely isolated heart in which the dynamics of both ventricles could be controlled. The blood from the right heart was aerated by means of an artificial lung consisting of a rapidly rotating bakelite disc which sprayed the blood in a thin film on to the sides of a large glass reservoir. The coronary arteries were cannulated and perfused with defibrinated dogs' blood under constant pressure and temperature, the cannulae being

inserted into the right circumflex, left circumflex, and anterior left descending arteries close to their points of origin (fig. 1A). The blood from the coronary system was drained through a Morawitz cannula in the coronary sinus.

The assembly used is shown diagrammatically in figure 2, and the component parts are described in the legend beneath the figure. The method developed for measuring coronary inflow at constant pressure was as follows: the graduated perfusion reservoir, *C*, was filled about half full of blood by means of pump *F*; then the large air tank, *A*, in communication with it was pumped up to the desired perfusion pressure (usually 100 to 120 mm. Hg). As blood was forced out of the reservoir into the coronary arteries, it was constantly replaced by pump *F*, and by adjusting screw clamp *Y* the level in the reservoir was maintained constant within 1 cm. Each stroke of the pump (15 strokes per min.) added from 5 to 20 cc. of blood to the reservoir, depending on the adjustment of clamp *Y*, which was determined in turn by the rate of coronary inflow. But since the total air volume of the tank, *A*, and the upper part of the perfusion reservoir was so large (55 liters), the introduction of 20 cc. of fluid into the system changed the pressure only slightly—less than 0.5 mm. Hg. Since the hydrostatic level in the perfusion reservoir was kept constant within 1 cm. during an experiment, the change in hydrostatic pressure could be kept constant within 1 mm. Hg. When a reading of the total coronary inflow was to be taken, stopcock *W* was turned so as to cut off the perfusion reservoir from the pump, and the fall of the blood level between graduations representing 10 cc. (a drop of 10 mm. which is less than 1 mm. Hg) was timed with a stopwatch.

Coronary sinus outflow was measured by catching the blood coming from the Morawitz cannula in a graduated cylinder, using stopcock *J* to divert the blood from the collecting reservoir to the graduate and timing it with a stopwatch. Aortic and pulmonary artery flows were measured in the same way, using stopcocks *L* and *K* respectively.

Blood to the right heart (and also to the left heart in the isolated heart preparations) was supplied from the main blood reservoir *B*. The venous inflow pressures were regulated by screw clamps *R* and *S* and read in manometers *Q*. The pressures within the ventricular chambers were controlled by changing the amount of the artificial resistances. The temperature in the preparation was maintained constant by enclosing the entire apparatus in a cabinet electrically heated and thermostatically controlled. The heart was either allowed to beat at its own rhythm or else driven at a constant rate by electrical stimuli applied to the right auricle from an inductorium and Lewis interrupter.

Special precautions were necessary in the preparation of the blood for perfusion. Usually 2 or 3 large dogs were anesthetized with ether and

bled to death from the carotid artery. The blood was defibrinated by shaking with glass beads, mixed well, and then passed twice through the

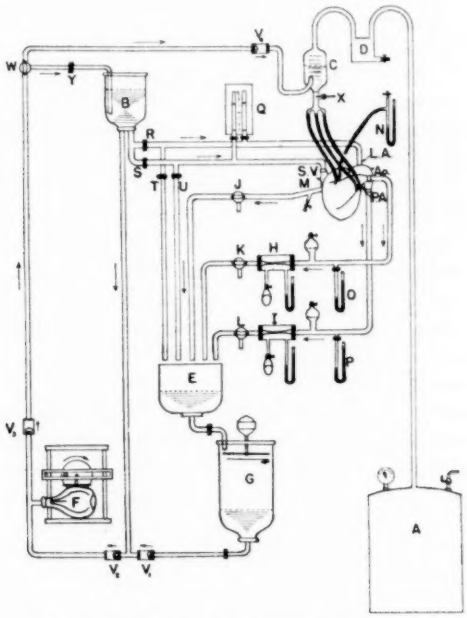

Fig. 2. Diagram of apparatus used for isolated heart preparation. *A*, compressed air tank (approx. 55 liters cap.); *B*, main blood reservoir; *C*, graduated constant pressure coronary perfusion reservoir; *D*, fluid trap in air line; *E*, collecting reservoir; *F*, pump: rubber bulb rhythmically compressed by motor; *G*, blood aerator; *H* and *I*, artificial peripheral resistances for right and left hearts respectively; *J*, 3-way stopcock for collecting coronary sinus outflow; *K* and *L*, 3-way stopcocks for collecting blood from right and left hearts respectively; *M*, Morawitz cannula in coronary sinus; *N*, Hg manometer for measuring coronary infusion pressure; *O* and *P*, Hg manometers for measuring pulmonary arterial and aortic pressures respectively; *Q*, blood manometers for measuring right and left venous pressures; *R* and *S*, screw clamps for regulating left and right venous inflows respectively; *T* and *U*, screw clamps on bleeder tubes for rapidly lowering venous pressures; V_1, V_2, V_3 and V_4, one way valves; *W*, 3-way stopcock for cutting off reservoir *C* from pump; *X*, thermometer in coronary inflow tube; *Y*, screw clamp for adjusting distribution of blood from pump between reservoirs *B* and *C*; *Ao*, aorta; *P. A.*, pulmonary artery; *L. A.*, inflow tube into left auricle; *S. V.*, superior vena cava.

When a heart-lung preparation was used, tube *L.A.* and the tube system *P.A.–H–K* were not used.

inflated lungs of one of the dead dogs. This step, experience showed, was necessary to remove a strong vaso-constrictor substance which was almost

invariably present in defibrinated blood not so treated. The blood was also filtered 5 times through fine mesh cambric (50μ) to remove any possible traces of fibrin. About 100 mgm. of heparin were added to the blood (3 liters) after it was placed in the reservoir to prevent clotting when the defibrinated blood mixed with the heparinized blood of the experimental animal. It was also found that the heart was maintained in better condition when 2 grams of glucose and 10 cc. of 10% calcium gluconate solution (the latter kindly supplied to us by the Sandoz Chemical Works, Inc.) were added to the perfusing blood.

The three coronary arteries were cannulated in turn after the heart-lung or isolated heart had been prepared. Care was taken to insert the cannula in the anterior descending coronary artery high enough to insure a blood supply to its septal branch, and this was always checked postmortem.

In this preparation, then, we had a completely denervated isolated heart (or heart-lung) beating at a constant rate and supplied with coronary blood at constant temperature and pressure. Keeping these factors constant, the arterial and/or venous pressures on one or both sides of the heart were varied over wide ranges so as to change the magnitude of the compressing force exerted by the contracting heart muscle on the intramural coronary vessels. We were interested in the effect of such procedures on the total coronary inflow, the coronary sinus outflow, and the percentage relationship between these two flows.

Results. It is apparent from the foregoing brief description that this was a particularly difficult preparation to make, and it is therefore not surprising that we succeeded in completing only 11 experiments out of 40 trials. Of these 11 experiments, 7 were rejected, either because postmortem examination showed leakage around the tip of the Morawitz cannula, occlusion of one of the coronary arteries, failure to supply blood to important branches of the coronary arteries, or because one or more of the important factors (arterial and venous pressure, coronary perfusion pressure, heart rate and temperature) could not be adequately controlled. As far as these incomplete experiments went, they were all in accord with the 4 entirely successful ones reported in table 1 and the 5th shown in table 3.

Experiments 3 and 5 (table 1) were both done on heart-lung preparations. In experiment 3, at an initial aortic mean pressure of 46 mm. Hg (compared with a coronary pressure of 110 mm. Hg) and a pressure of 5.8 cm. of blood in the superior vena cava, the total coronary inflow started at 68 cc./min. and rose gradually to 75 cc./min. At the same time the coronary sinus outflow varied from 33 to 37 cc./min. The sinus flow was thus 45 to 49 per cent of the inflow. At this point the mean intramuscular tension of the heart was increased by raising the aortic pressure to 96 mm. Hg and the superior vena cava pressure to 11.7 cm. of blood. The coronary inflow

then dropped from 75 to 55 cc./min., and simultaneously the sinus outflow increased from 37 to 59 cc./min., 4 cc./min. more than the total inflow, making the sinus outflow 107 per cent of the total coronary inflow. When the aortic and venous pressures were decreased to their original levels, the coronary inflow rose to 60 cc./min. and the outflow fell to

TABLE 1

Effect of changing intramuscular tension and intracardiac pressure on coronary flow

	TIME	CORONARY INFLOW	CORONARY SINUS OUTFLOW	SINUS OUTFLOW/CORONARY INFLOW	SYSTEMIC ARTERIAL PRESSURE	SYSTEMIC VENOUS PRESSURE	PULMONARY ARTERIAL PRESSURE	PULMONARY VENOUS PRESSURE
	minutes	*cc./minute*	*cc./minute*	*per cent*	*mm. Hg*	*cm. blood*	*mm. Hg*	*cm. blood*
Expt. 3. Heart-lung. Coronary pressure 110 mm. Hg. Heart rate 144/min.	0	73	33	45	46	5.8		
	2	75	37	49	46	5.8		
	5	55	59	107	96	11.7		
	8	60	27	45	46	5.8		
Expt. 5. Heart-lung. Coronary pressure 120 mm. Hg. Heart rate 120–156/min.	0	97	21	22	72	2.5		
	2	100	14	14	72	3.5		
	5	81	43	53	124	7.5		
	6	81	38	47	118	7.5		
	7	111	9	8	24	3.5		
	10	120	10	8	24	3.5		
	11	73	23	31	134	12.5		
	12	60	23	38	134	12.5		
Expt. 17. Isolated heart. Coronary pressure 130 mm. Hg. Heart rate 120–144/min.	0	125	45	36	20	13.5	0	15.5
	2	115	81	71	150	21	60	25
	8	100	39	39	0	16.5	10	14
	12	94	61	65	130	21.5	40	26
Expt. 19. Isolated heart. Coronary pressure 120 mm. Hg. Heart rate 125/min.	0	167	86	52	96	2.5	15	13.2
	3	150	107	72	156	10	60	20.2
	8	200	62	31	16	0	5	5.2
	12	125	115	92	126	15	55	20
	15	167	43	26	6	0	0	2.2
	19	120	64	53	71	2.5	11	13.2

27 cc./min., the latter being again 45 per cent of the former. This change in flow occurred at a constant coronary pressure of 110 mm. Hg, a constant temperature of 37°C., and with the heart driven constantly at a rate of 144/min.

In experiment 5, although the heart rate varied irregularly from 120

to 156/min., changing the mean intramuscular tension of the heart affected the coronary flows in the same way, viz.: raising the aortic and vena cava pressures decreased the inflow and increased the sinus outflow, thus increasing the sinus outflow/coronary inflow ratio, and this was reversed again on lowering the pressures in the aorta and vena cava.

The variations in heart rate in this experiment occurred because it was impossible to cause the heart to follow the regular stimuli from the Lewis interrupter. In order to see what effects heart rate changes alone might produce, three experiments were done in which only the heart rate was varied, all other variables being maintained constant. In all of these the changes in heart rate produced no significant effect on the coronary inflow and sinus outflow or on the sinus outflow/coronary inflow ratio. Table 2 shows the results of one of these experiments.

TABLE 2

Effect of changing heart rate on coronary flow

Experiment 17. Isolated heart—coronary pressure, 130 mm. Hg

TIME	HEART RATE	CORONARY INFLOW	CORONARY SINUS OUTFLOW	SINUS OUTFLOW/ CORONARY INFLOW	SYSTEMIC ARTERIAL PRESSURE	SYSTEMIC VENOUS PRESSURE	PULMONARY ARTERIAL PRESSURE	PULMONARY VENOUS PRESSURE
minutes	*beats/ minute*	*cc./ minute*	*cc./ minute*	*per cent*	*mm. Hg*	*cm. blood*	*mm. Hg*	*cm. blood*
0	114	94	57	61	140	21	40	26
1	144	94	61	65	130	21.5	40	26
2	144	100	59	59	130	23	40	26
4*	174	100	58	58	90	21.5	20	26.5

* The arterial pressures could not be maintained at the former levels at this heart rate.

Experiments 17 and 19 of table 1 present results obtained on completely isolated hearts when the pressure in the pulmonary vessels as well as in the systemic were under control. In both of these experiments, the aortic and pulmonary arterial pressures, and the venous pressures in both the right and left auricles were all raised or lowered simultaneously to change mean cardiac intramuscular tension. The effects on the coronary flows were exactly the same as in the heart-lung preparation. Increasing mean cardiac intramuscular tension of the heart decreased the total coronary inflow, increased the sinus outflow and increased the sinus outflow/coronary inflow ratio; and these changes were reversed again when the mean intramuscular tension of the heart was lowered. In experiment 17 the sinus outflow/coronary inflow ratio varied from 36 per cent to 71 per cent, and in experiment 19 it varied from 26 per cent to 92 per cent.

In a fifth experiment, shown in detail in table 3, two changes in procedure were made: 1, the pressures on the inflow and outflow sides of the right heart were changed separately from those on the left to permit more detailed analysis of the effects, and 2, the cannulation of the left coronary

TABLE 3

Summary of experiment 61, an isolated heart preparation—coronary pressure, 120 mm. Hg

	TIME	CORONARY INFLOW	CORONARY SINUS OUTFLOW	SINUS OUTFLOW/CORONARY INFLOW	SYSTEMIC ARTERIAL PRESSURE	SYSTEMIC VENOUS PRESSURE	PULMONARY ARTERIAL PRESSURE	PULMONARY VENOUS PRESSURE	HEART RATE
	minutes	*cc./minute*	*cc./minute*	*per cent*	*mm. Hg*	*cm. blood*	*mm. Hg*	*cm. blood*	*beats/minute*
A	0	182	127	70	10	1.0	2	6.5	84
	1½	176	124	70	10	1.0	0	6.5	84
	2¼	182	126	69	10	1.0	0	6.5	84
	4	176	124	70	10	1.0	0	6.5	84
B	6	146	95	65	121	13.0	37	11.5	
	8½	158	110	70	106	17.0	36	17.5	88
	9½	140	98	70	116	15.0	36	11.5	84
C	11½	150	120	80	106	16.0	36	22.5	92
	12½	143	114	80	116	15.0	32	22.5	92
	13½	140	115	82		13.0		20.5	92
	15	143	118	82	106	17.0	32	22.5	92
	16	146	117	80		16.0		22.5	92
D	18	102	61	60	96	17.0	0	6.5	84
	19	113	62	55	86	17.0	0	6.5	84
	20	97	56	57	86	17.0	0	7.0	84
	21¼	90	54	60	86	17.0	0	7.0	84
E	23	167	116	70	4	1.0	0	6.5	84
	25	140	84?	60	0	1.0	0	6.5	84
	27	110	76	70	0	1.0	0	6.5	84
	28½	98	64	65	0	1.0	0	6.5	84
F	30½	80	62	77	0	1.0	32	22.5	96
	32	75	56	75	0	1.0	30	22.5	96
	34	77	58	75	0	1.0	30	22.5	96
	35½	69	53	77	0	1.0	28	22.5	96
G	37	67	52	77	96	17.0	32	22.5	96
	38½	67	53	80	86	17.0	32	22.5	96
	40	67	52	77	90	18.0	30	22.5	96
	Coronary pressure lowered from 120 to 75 mm. Hg								
H	45	19	25	130	76	17.0	32	21.5	92
	46½	19	23	120	61	17.0	32	21.5	84
	48¼	18	24	130	36	17.0	32	21.5	80
	51	19	25	130	54	17.0	32	21.5	80
I	52½	25	26	105	2	1.0	30	22.5	80
	54½	23	25	105	0	1.0	26	22.5	84
	56	24	26	105	2	1.0	28	22.5	84

TABLE 3—*Concluded*

	TIME	CORONARY INFLOW	CORONARY SINUS OUTFLOW	SINUS OUTFLOW/ CORONARY INFLOW	SYSTEMIC ARTERIAL PRESSURE	SYSTEMIC VENOUS PRESSURE	PULMONARY ARTERIAL PRESSURE	PULMONARY VENOUS PRESSURE	HEART RATE
	minutes	*cc./ minute*	*cc./ minute*	*per cent*	*mm.* Hg	*cm. blood*	*mm.* Hg	*cm. blood*	*beats/ minute*
J	59	26	13	50	2	6.0	0	6.5	84
	60	26	13	50	0	6.0	0	6.5	80
	62½	29	17	60	0	6.0	0	6.5	80
	64½	30	15	50	4	6.0	0	6.5	82
	67½	32	21	65	4	6.0	0	6.5	82
	68	31	18	60	4	6.0	0	6.5	80
K	69½	21	26	125	76	18.0	32	22.5	84
	71	24	27	115	56	18.0	30	22.5	72
	72½	22	27	125	56	18.0	30	22.5	72
	75	22	27	125	80	18.0	30	22.5	
L	77½	36	22	60	4	6.0	0	5.5	72
	80½	32	19	60	0	6.0	0	6.5	72
	82½	29	17	60	0	6.0	0	10.5	72
	84	28	16	55	0	6.0	0	10.5	
	85	Heart deliberately fibrillated by electrical stimulation							

artery was altered as shown in figure 1 B. The left coronary artery was dissected free up to its emergence from the aorta. Two coronary perfusion cannulae were then inserted in the branch-free portion of the left circumflex artery, one facing toward its origin so as to feed the left descending, the main left coronary artery was tied off close to its mouth. The results in this experiment are in accord with the previous ones. The early part of the experiment where the coronary inflow pressure was high (A to G) is difficult to analyze because of the progressive decrease in coronary flow which levels off in the latter part after G. Nevertheless, the early part is shown to illustrate another difficulty which caused us to discard some of the 7 incomplete experiments. The only information to be obtained from it is the fluctuation in the sinus outflow/coronary inflow ratio. In C (table 3) the arterial and venous pressures (and consequently mean cardiac intramuscular tension and mean intracardiac pressure) in both right and left hearts were high, and the sinus outflow was 80 to 82 per cent of the total inflow; in A and E when all pressures were low, it was 69 to 70 per cent and 60 to 70 per cent respectively. That the intramuscular tension in the right heart and the mean intracardiac pressure of this side were chiefly responsible for the effect is shown in the part of the experiment where the pressures in the right and left ventricles were varied independently. When the pressures on the left side were high and those on the right low, the sinus outflow/coronary inflow ratio was about the

same as when the pressures on both sides were low (compare 55 to 60 per cent in D with 69 to 70 per cent in A and 60 to 70 per cent in E). When the pressures on the left were low and those on the right high, the sinus outflow/coronary inflow ratio was about the same as when all pressures were high (compare 75 to 77 per cent in F with 80 to 82 per cent in C and 77 to 80 per cent in G).

The inverse relationship between total coronary inflow and mean cardiac intramuscular tension is suggested by 1, the sharp drop in total coronary inflow between A and B (from 176–182 cc./min. to 140–158 cc./min.), when the pressures in both ventricles were raised; and 2, the rise in total coronary inflow between C and E when all pressures were lowered. This rise in flow occurred despite the general tendency for the coronary inflow

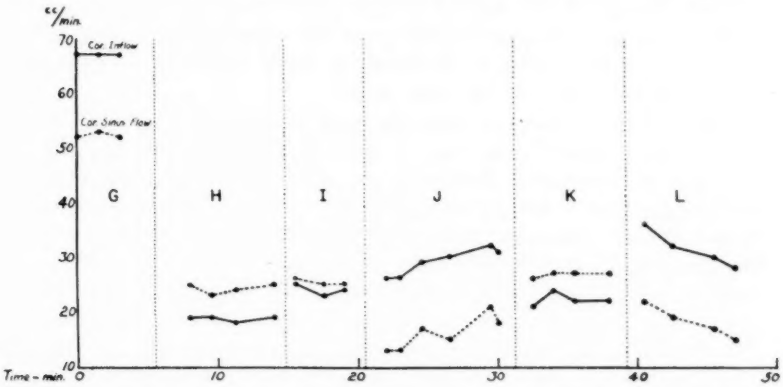

Fig. 3. Graph showing time curves of coronary inflow and sinus outflow in latter half of experiment 61. Discussed in text.

to fall off. The fact that the increase occurred when the mean intramuscular tension of the left ventricle was dropped, E, and not when that of the right was lowered, D, points to the greater effect of the left ventricle than the right on the coronary inflow.

A striking change in coronary flow was produced in this experiment when the mean intramuscular tension of the heart was maintained at a high level but the coronary inflow pressure was dropped from 120 to 75 mm. Hg. As might be expected, a sharp drop in coronary flow[3] occurred but the sinus outflow now exceeded the total coronary inflow, the ratio between the two being 130 per cent (compare G and H, table 3 and fig. 3).

[3] A number of other experiments were done in which the coronary pressure was changed while leaving the other factors unchanged, and in every instance a direct correlation between the magnitude of the coronary inflow and the height of the coronary pressure could be demonstrated. This is in accord with the results of Anrep and King (6).

With the coronary inflow pressure now established at this new level, the effects of changes in mean cardiac intramuscular tension were clearly shown. Lowering the pressures in the left ventricle had practically no effect on the sinus outflow but increased the total coronary inflow (compare H and I, table 3 and fig. 3), the sinus outflow remaining above the total inflow. Now, lowering the pressures in the right heart increased the total coronary inflow further (compare I and J) about as much as the same procedure on the left heart had done, but the sinus outflow decreased sharply and became only 50 to 65 per cent of the total inflow. When H, J, K and L are compared, it will be seen that *a*, the inhibiting effect of augmenting intramuscular tension in the two ventricles on total coronary flow is reversible; *b*, the augmenting effect on the sinus outflow of raising the intramuscular tension in the right heart is reversible; *c*, the ability to obtain a persistent flow from the coronary sinus greater than the total coronary inflow is reproducible under conditions of high mean intramuscular tension in the right heart.

The inverse relationship between mean cardiac intramuscular tension and coronary inflow is also seen in table 4, which presents the effect of spontaneous ventricular fibrillation on coronary inflow in the isolated heart preparation. Inspection of this table will show that an increase in coronary inflow following ventricular fibrillation as described by Anrep and Häusler (5) occurs only when the pressures in both ventricles are definitely elevated, to begin with viz: exper. 15, 19, and 20. Fibrillation of the ventricles under these circumstances would drop cardiac intramuscular tensions to zero when the venous inflows were stopped and so decrease the compression of the intramural coronary vessels. Experiment 17 does not show this expected increase because the natural decrease in coronary flow masked the effect. In experiment 21 the pressures in the heart cavities were practically zero before fibrillation, so there was no change in the compression of the coronary vessels and as expected no change in coronary flow.

A further type of experiment which we performed demonstrates this retardation of coronary flow. In a preparation with ventricular fibrillation, rhythmic manual squeezing of the heart at the rate of 170 times/min. reduced the coronary inflow to 37 cc./min. as compared with control flow values before and after the massage of 61 and 59 cc./min. respectively. At the same time this massage increased the arterial pressure from 0 to 20 mm. Hg.

Discussion. The effects of coronary inflow pressure on total coronary inflow found in this preparation are in accord with the observations of Anrep and King (6).

The effects of changing heart rate on total coronary inflow found in this preparation do not agree with those reported by Rein (14) or Anrep and

Häusler (5). In their experiments other variables must have been introduced which could modify the driving pressure, the dynamics of the heart beat or actively alter the calibre of the coronary vessels. When these complications are prevented, our results show that coronary flow

TABLE 4

Effect of spontaneous ventricular fibrillation on coronary inflow

EXPERIMENT	STATE OF HEART	TIME	CORONARY INFLOW	SYSTEMIC ARTERIAL PRESSURE	SYSTEMIC VENOUS PRESSURE	PULMONARY ARTERIAL PRESSURE	PULMONARY VENOUS PRESSURE
			cc./minute	*mm.* Hg	*cm. blood*	*mm.* Hg.	*cm. blood*
15	Beating heart	0	48	40	23	20	23
		3	48	40	24.5	20	23
	Ventricular fibrillation	6	56	0	0	0	0
		8	71	0	0	0	0
		12	79	0	0	0	0
17	Beating heart	0	54	15	18.5	0	18
		6	38	15	14	0	15
	Ventricular fibrillation	10	24	0	0	0	0
		11	21	0	0	0	0
19	Beating heart	0	111	16	1.7	7	9.2
		2	107	16	1.5	7	9.2
		4	103	16	1.2	7	8.7
	Ventricular fibrillation	8	125	0	0	0	0
		10	120	0	0	0	0
20	Beating heart	0	125	30	27.5	20	25.5
		1	111	30	26	20	27.5
		2	107	30	27	20	26
	Ventricular fibrillation	5	120	0	0	0	0
		6	130	0	0	0	0
21	Beating heart	0	32	0	0.5	0	0
		1	30	0	0.5	0	0
	Ventricular fibrillation	2	30	0	0	0	0
		6	28	0	0	0	0

is independent of the rate at which the heart beats. The inference must follow that under these circumstances the changes in flow during systole must be compensated for during diastole.

The decrease in total coronary inflow found in this preparation when the

venous return and arterial resistance is increased is to be expected on a priori grounds. Rein (14), Essex and his co-workers (8) and Klisiecki and Flek (13), however, obtained exactly the reverse results under such circumstances in the intact animal. Apparently in the intact animal other factors come into play which neutralize the mechanical effect of increase in the dynamic activity of the heart. What these are we are not prepared to say. However, our results do not support Rein's argument that local metabolic products liberated when the activity of the heart is increased are responsible for the increased coronary flow found in their experiments, since we obtained a decreased coronary flow under these conditions in our experiments with the isolated heart. Here the action of local products is either ineffective or over-balanced by the direct mechanical inhibiting action of the increased vigor of the heart. Rein's results must therefore be attributed in part at least to adjustments propagated via the nerves or by humoral transmission.

In the isolated heart preparation, our results indicate that conditions exist in which the coronary flow varies inversely with the work that the heart is doing (roughly calculated as the product of outflow and mean pressure existing in the aorta and pulmonary artery) and presumably also with its energy consumption. This is a vicious mechanism.[4]

Examination of experiments reported in the past by Anrep and Häusler (4) and Wiggers (18, 19) with perfused single coronary arteries shows a direct relationship between the power of the heart and coronary inflow similar to that reported by us, but their experiments are not convincing since the results obtained might have been due to a readjustment of flow between the cannulated coronary artery and those not cannulated.

Changing the venous and arterial pressures also affects coronary flow by changing the pressure in the heart cavities and hence the pressure acting on the coronary blood as it leaves the coronary vessels to enter these heart cavities. The intracardiac pressure, expecially that within the right heart, will in this way affect the total coronary inflow also. In this connection, it is interesting that on several occasions it was found that clamping the coronary sinus cannula, as might be expected, decreased the total coronary inflow.[5] In our preparation, however, the mean pressure

[4] It is possible that a similar vicious mechanism comes into operation in the intact animal on excessive exertion or in acutely developing hypertension when the coronary vessels are not diseased, and on ordinary moderate exertion when the coronaries are diseased.

[5] In a recent experiment with the isolated beating heart preparation (Katz and Mendlowitz, this Journal, 1938, this issue), this decrease in coronary inflow following coronary sinus obstruction was confirmed. In this experiment, the sinus obstruction led to failure of the heart; releasing the sinus obstruction was followed by rapid recovery of the power of the heart. In view of these observations, it is difficult to see the rationale of coronary sinus ligation as a procedure to be employed to increase the coronary arterial bed.

of the blood leaving the coronary sinus does not fluctuate significantly since the Morawitz cannula opens to atmospheric pressure. In the intact animal with the coronary sinus entering into the right auricle, the pressure in the right heart cavities will affect the pressure in the coronary sinus, as well as that in the Thebesian channels.

The sinus outflow is determined by the same factors which determine coronary inflow, namely, the mean intramuscular tension of the heart walls and the mean pressure within the heart cavities. However, since the sinus is only one of the portals of exit, fluctuations in its flow will vary independently of the total coronary inflow because of redistribution of flow between it and the Thebesian channels. In our experiments, this is exaggerated by the fact that the sinus opens to air and so does not share in the fluctuation of pressure which exist at the mouths of the Thebesian vessels. Recently, following up our preliminary report, Johnson and Wiggers (11) have shown that fluctuations in sinus outflow in the open chested animal similar to those found by us occur when the sinus is permitted to empty into the vena cava. Presumably, therefore, redistribution of the coronary outflow between the sinus and the Thebesians occurs also in the intact animal. Our results show clearly that coronary sinus flow measurements as obtained with the Morawitz cannula are no certain measure of total coronary inflow but often may indicate the redistribution of outflow between the sinus and the Thebesian channels. Results utilizing this method to determine total coronary flow are thus subject to serious error and must be accepted with considerable reservation.

We are unable to substantiate the results of Evans and Starling (9) and Anrep et al. (3) that the coronary sinus outflow is about 60 per cent of the total coronary inflow. In our hands with this preparation the sinus outflow formed anywhere from 8 to 130 per cent of the total inflow and in single preparations varied from 45 per cent to 107 per cent in experiment 3, 8 per cent to 53 per cent in experiment 5, 36 per cent to 71 per cent in experiment 17, 26 per cent to 92 per cent in experiment 19, and 50 per cent to 130 per cent in experiment 61. Therefore results concerning the action of drugs, of nerves and of other factors based on coronary sinus outflow as an index of total coronary flow must be cautiously interpreted—a procedure not followed in the past.

Not only does the coronary sinus drain that portion of the total coronary arterial flow not drained by the Thebesian channels, but it may, as these experiments demonstrate, drain blood *entering* the coronary system via the Thebesians. After all, no valves have been demonstrated to exist in the coronary system, and when the pressure gradients are reversed, the flow in these channels will be reversed. This fact which we have demonstrated previously (Bohning, Jochim and Katz, 7) is confirmed by the present experiments. Stella's (16) results to the contrary are not valid,

as we have previously pointed out, because his method of study leads to the abolition of effective heart action after a few beats.

The present experiments leave no doubt that the Thebesian vessels can become irrigation instead of drainage channels when the pressure gradient existing between the coronary arteries and the heart cavities is reversed. Thus, there will develop an ebb and flow in the Thebesian channels during the heart cycle with the back flow occurring during diastole. As the pressure within the right heart cavity rises, the back flow in the Thebesian channels will tend to approach and then exceed the forward flow. Eventually, the magnitude of the back flow will become sufficient to cause the sinus outflow to exceed the coronary artery inflow.

Previous work both anatomical and experimental (cf. Wearn, 17; and Anrep, Blalock and Hammouda, 3, for early literature) and some recent observations of our own (Katz, Jochim and Weinstein, 12) have shown that most of the Thebesians open into the right side of the heart. It is here that the Thebesian channels will become the important portals for nourishing the heart when the normal channels, the coronary arteries, are obstructed. In the left ventricle the paucity of these channels will prevent them from compensating adequately for impairments in coronary inflow. It would be expected on this basis that infarction would be much more frequent in the left ventricle than in the auricles or the right ventricle, and this is found to be the case in surveying human autopsy material (cf. Saphir, Priest, Hamburger and Katz, 15). Even in the auricles and in the right ventricle infarction would occur more readily if the Thebesian openings were blocked as happens where a mural thrombus is located.

SUMMARY

A method is described for measuring total coronary inflow and coronary sinus outflow in a completely denervated heart-lung or isolated heart preparation in which the coronary arteries are perfused with blood at constant pressure and all other variables are controlled. The following were the chief results obtained in this preparation:

1. Changes in heart rate alone do not appreciably alter the rate of the total coronary blood flow.

2. The rate of total coronary blood flow varies directly with the coronary perfusion pressure, other conditions being constant.

3. When all other variables are kept constant, the total coronary inflow is decreased by raising the mean pressure within the heart cavities and thus the mean intramuscular tension within their walls. Decreasing these pressures increases the total coronary inflow. This change in coronary inflow can be effected by varying the pressures and tensions of each side of the heart alone.

4. When the mean pressure of the heart cavities is changed, the rate of sinus outflow varies in a direction opposite to the total coronary inflow,

so that the ratio between the two varies widely. Changes in sinus outflow depend almost entirely on changes in pressure in the right side of the heart, the pressures in the left side having little effect.

5. The coronary sinus outflow may persistently exceed the total coronary inflow when the mean pressure in the heart cavities and the mean intramuscular tension of their walls are high relative to the coronary perfusion pressure; changes in the pressure in the right heart are more effective than in the left in this regard.

The significance of these findings is discussed. It is pointed out among other things 1, that the coronary sinus outflow cannot be used as a measure of the total coronary flow, and 2, that significant passive changes in the calibre of the coronary vessels are produced by altering the extravascular tension in the heart walls and that this factor as well as variations in the aortic pressure must be ruled out before changes in coronary inflow can be ascribed to active changes in the tone of the muscles in the coronary vessel walls.

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THE DISTRIBUTION OF THE CORONARY BLOOD FLOW[1,2]

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While it is generally known that anatomical variations exist in the coronary vascular tree in different individuals of the same species, it is not clearly established how great the variations are in terms of partition of the blood flow; in fact, there has been a tendency to minimize the variations in this partition. For example, Evans and Starling (1), Markwalder and Starling (2), Morawitz and Zahn (3) and more recently Anrep, Blalock, and Hammouda (4) have reported that the coronary sinus flow obtained with a Morawitz cannula is a constant fraction of the total coronary blood flow (approximately 60 per cent), and Anrep and his associates have insisted that this ratio is the same regardless of whether the ventricles are beating rhythmically, are at a standstill, or are fibrillating (4). This was not substantiated by Wearn (5) or by ourselves (Katz, Jochim and Bohning, 6). Wearn found a wide variation in the proportion of total coronary flow drained by the coronary sinus on perfusing human hearts postmortem. We (6) have shown that both in the heart-lung preparation and in the isolated heart in which the coronaries are perfused under constant pressure, changing the power of the heart beat by altering the venous and arterial pressures leads to wide variations in the proportion of blood drained by the coronary sinus. The range in the ratio of coronary sinus outflow/total coronary inflow obtained by us was from 8 to 130 per cent.

The majority of workers dealing with partition of outflow have considered only the partition between the sinus and all other remaining channels. The only attempt to differentiate between drainage channels of the right and left heart of which we are aware was made by Wearn (5). He distinguished between drainage into the right and left heart cavities in the human heart perfused postmortem and found that there was a wide variation in the relation of the two; in some the drainage in the left heart

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[2] A preliminary report of this work was presented before the American Physiological Society in 1935.

was as great as in the right. This is surprising since it is generally accepted that the accessory veins and Thebesian channels drain chiefly into the right heart, and our results point in the same direction.

In the present report we are presenting our results on the pattern of the coronary flow bed. This we have analyzed as follows: 1, the relative outflow from *a*, the coronary sinus; *b*, the accessory veins and Thebesian channels of the right heart, and *c*, the Thebesian channels of the left heart; 2, the relative proportion of total inflow in each of the three main coronary arterial branches viz., the left circumflex, the anterior left descending, and the right circumflex, and 3, the relative contribution of

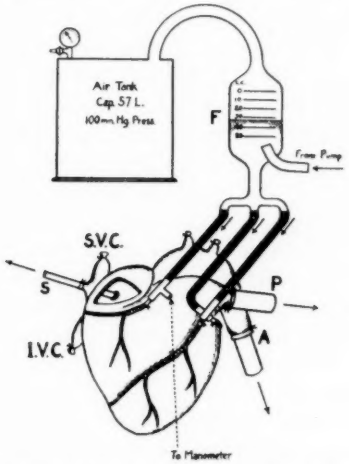

Fig. 1. Diagram showing the special coronary inflow meter and the connections to heart. Discussed in text.

each of these main arterial branches to the three channels of outflow mentioned above in 1.

METHOD. All 12 experiments were carried out on the isolated heart with ventricles fibrillating and with the coronaries perfused with defibrinated dogs' blood at constant pressure (120 mm. Hg) and constant temperature. The right circumflex, left circumflex, and anterior left descending branches of the coronary arteries were dissected free and cannulated after a "physiological" heart-lung preparation was made by tying off all the systemic veins and arteries coming off from the heart. Heparin was used to render the blood noncoagulable. To ensure a viable preparation, the ventricles were permitted to beat until all the cannulae were inserted; then they were thrown into fibrillation. (Occasionally, the heart would fibrillate before this was accomplished. Under these circumstances the

fibrillating heart was fed via the aorta until the cannulation was completed.) The connections to the heart are shown diagrammatically in figure 1. In the first nine experiments the cannulae were inserted as shown in figure 1 a, and in the last 3 as in figure 1 b of our previous report (6). No difference in results with the 2 methods of cannulation was noted. Coronary sinus flow was measured by means of a Morawitz cannula (*S*, fig. 1); blood flow from the accessory veins and the Thebesian channels of the right heart was measured by means of a cannula in the main pulmonary artery, *P*, inserted after the coronary cannulation was completed; blood flow from the Thebesian channels of the left ventricle was measured by means of a cannula, *A*, in the descending aorta. The method of measuring coronary inflow and the general technique are described in our previous report (Katz, Jochim and Bohning, 6).

Simultaneous measurement of the outflow from the coronary sinus, pulmonary artery, and aorta gave the total coronary outflow, which was always equal, within experimental error, to the total inflow as measured with the special inflow flowmeter (F). From these readings the proportion of the total flow draining from each of these outflow channels could be calculated easily. The portion of flow carried by each of the three arteries was determined by measuring the changes in inflow after the other two branches were clamped. The proportion of blood drained from these arterial branches by each of the three outflow channels could be measured at the same time. To avoid the deleterious action of prolonged anoxemia, the clamping was maintained just long enough to permit several stable readings to be made.

Discussion of results. Table 1 shows the distribution of total inflow between the three main coronary branches. These were calculated in per cent, calling 100 per cent the value of the total flow which would have existed at the time of the occlusion, had the flow at this time been measured. This was calculated for each occlusion by plotting on a time scale the flow before the occlusion and the stabilized flow after the occlusion, connecting the two by a straight line, and interpolating the probable value for total flow at the time when the readings through the single artery were made. This procedure was found necessary for two reasons: *a*, the flow was not always constant during an experiment, but often showed a progressive falling off, and rarely a gradual increase; *b*, the anoxemia with occlusion caused a temporary acceleration of flow following release which took several minutes to disappear. The partition of flow between these three branches is not constant but quite variable in the different preparations. On the average the left coronary artery carries 82 per cent of the total flow, and the right 18 per cent, but in individual cases the portion carried by the left varied from 69 per cent to 92 per cent and that by the right from 8 per cent to 31 per cent. The left circumflex carried on the

average 46 per cent, and the anterior left descending 41 per cent of the total inflow; however, in individual cases the ratios were not so nearly equal but ranged from 25 per cent in the left circumflex and 68 per cent in the anterior left descending in experiment 3, to 60 per cent and 27 per cent respectively in experiment 12. The variability in our experiments is much greater than that reported by Anrep and his associates (4) in four animals, although the averages are of the order reported by them. We cannot agree that the distribution of blood between the three coronary arteries is in any sense constant in a series of individuals of the same species.

When the sum of the flows of the three coronary arteries obtained by adding up the percentage of total flow going through each is computed,

TABLE 1

Percentage of total inflow carried by different coronary arterial branches

EXPT. NO.	LEFT CIRCUMFLEX ARTERY	ANTERIOR LEFT DESCENDING ARTERY	RIGHT CIRCUMFLEX ARTERY	SUM OF THE FLOWS IN THE THREE BRANCHES COMPARED TO CONTROL FLOW (IN PER CENT)
1	37	36	31	104
2	50	25	20	95
3	28	68	23	119
4	46	62	14	122
5	51	35	20	106
6	47	39	22	108
7	42	51	13	106
8	47	30	16	93
9	53	41	14	108
10	40	41	10	91
11	47	39	19	105
12	60	27	8	95
Range	28-60	25-68	8-31	91-122
Av	46	41	18	105

it is found to average 105 per cent and range from 91 to 122 per cent. In part this is an expression of the experimental error in making the measurements and in part it is a measure of the amount of collaterals shared in common by two or more of these branches. Since we know of no reason why the sum of the individual flows should be less than 100 per cent, we can assume that 91 per cent instead of 100 per cent in experiment 10 is an experimental error. Therefore, the experimental error can be considered to be $\pm$ 9 per cent. Any value over 109 per cent would on this basis express the amount of functional collateral anastomoses. In two experiments, viz., experiments 3 and 4, values of 119 and 122 per cent were obtained indicating the probable presence of collaterals of the magnitude of

at least 10 and 13 per cent of total flow. If these two experiments are omitted from the calculations, the sums of the individual coronary flows become on the average 101 per cent instead of 105 per cent. These results suggest that in approximately $\frac{1}{6}$ of the animals, channels common to more than one artery existed which constituted a functional reserve equal to about 10 to 13 per cent of the total coronary flow. In the other $\frac{5}{6}$ of the animals these collateral channels were apparently absent. This is another variable in the pattern of the coronary flow bed.

The partition of the coronary outflow between the coronary sinus, the drainage channels of the right heart, and those of the left heart is shown in table 2. It will be seen that the partition is far from constant. The coronary sinus outflow constituted anywhere from 17 to 44 per cent (average

TABLE 2

Percentage of total outflow draining by way of various channels

EXPT. NO.	CORONARY SINUS	RIGHT VENTRICLE	LEFT VENTRICLE
1	44	45	11
2	26	72	2
3	28	50	22
4	39	61	0
5	42	58	0
6	17	69	14
7	27	62	11
8	38	51	11
9	37	43	20
10	31	56	13
11	25	67	8
12	24	62	14
Range	17–44	43–72	0–22
Av.	32	58	10

32 per cent) of the total outflow, and the other drainage channels anywhere from 56 to 83 per cent (with an average of 68 per cent). The low value of the coronary sinus outflow, which never approached 60 per cent, is due to the conditions existing when the living coronaries are perfused with the heart fibrillating. Under these conditions the pressures existing in the heart cavities and the tension in the heart muscle walls are close to zero, whereas in the normal beating heart these mean pressures and tensions are considerably above this value. Our recent results (Katz, Jochim and Bohning, 6) have shown that the sinus outflow/coronary inflow ratio varies inversely with the mean tension in the walls of the heart. Further evidence supporting this view is to be found in the data of table 3 in which the effect of throwing the ventricles into fibrillation on the sinus

outflow/coronary inflow ratio is correlated with the pressures existing in the heart cavities just before fibrillation was instituted. Thus it will be seen that the ratio was unchanged by fibrillation in experiment 13 where the mean cardiac pressures were zero before fibrillation, whereas a drop in the ratio from 54 per cent to 18 per cent and from 52 per cent to 34 per cent occurred in experiments 16 and 17 where the mean pressures within the heart were high before fibrillation. The other experiments with lower mean cardiac pressures before fibrillation show intermediate changes. The effect of the induction of fibrillation on the sinus outflow/coronary inflow ratio thus is definitely related to the dynamic state of the heart before ventricular fibrillation starts.

The results on outflow partition in the fibrillating heart are significant in that they show that the coronary sinus outflow under comparable conditions is a widely varying proportion of the total coronary inflow. The use of coronary sinus outflow as an index of the coronary flow is not justified. This confirms and extends our position previously published (Katz, Jochim and Bohning, 6) that total coronary flow cannot be determined from sinus outflow.

The proportion of coronary flow drained by the Morawitz cannula in these fibrillating experiments is more nearly the proportion of coronary flow leaving via the coronary sinus in the beating heart where the sinus is not cannulated than those obtained in the beating heart with the Morawitz cannula. In the fibrillating heart, the pressures at the mouths of the sinus and other drainage channels are approximately equal whereas in the beating heart the pressure at the mouth of the Morawitz cannula is definitely less than that at the mouths of the other drainage channels. The presence of a Morawitz cannula open to air in the beating heart tends to divert some of the blood normally draining into the heart cavities to the coronary sinus and thus makes its flow unnaturally large. It follows that the normal sinus flow in the beating heart is less in amount than previous work would indicate, its value being somewhere between 32 per cent of the total coronary flow found on the average in our experiments with the fibrillating heart and the 60 per cent found by others in the beating heart.

The distribution of drainage between the right and left hearts is quite variable although we found the flow into the right heart was always at least twice that into the left heart. The drainage into the right heart varied from 43 to 72 per cent (average 58 per cent). This was always greater than the sinus flow, and sometimes, as in experiment 6, as much as four times as great. The accessory veins and Thebesian channels are thus the major drainage channels in the fibrillating heart. The drainage into the left heart is small, varying from 0 to 22 per cent. While in some individuals it approaches the sinus flow, as in experiments 3 and 6, it never

approaches that of the right heart (contrary to some data given by Wearn). The importance of the relative distribution of the Thebesian channels

TABLE 3

Effect of ventricular fibrillation on the distribution of coronary outflow between the coronary sinus and other drainage channels

EXPT. NO.	SINUS OUTFLOW/CORONARY INFLOW		SYSTEMIC BLOOD PRESSURE		PULMONARY ARTERIAL BLOOD PRESSURE		SYSTEMIC VENOUS PRESSURE		PULMONARY VENOUS PRESSURE		CORONARY PERFUSION PRESSURE	HEART RATE BEFORE FIBRILLATION
	Beating heart	Vent. fib.	Beating heart	Vent. fib.	Beating heart	Vent. fib.	Beating heart	Vent. fib.	Beating heart	Vent. fib.		
	per cent	*per cent*	*mm. Hg*	*mm. Hg*	*mm. Hg*	*mm. Hg*	*cm. blood*	*cm. blood*	*cm. blood*	*cm. blood*	*mm. Hg*	*beats per min.*
13	37	37	0	0	0	0	0	0	0	0	120	80
14	39	33	11	0	0	0	3.5	0	5	0	150	114
15	23	17	16	0	7	0	1	0	9	0	125	114
16	54	18	36	0	15	0	14	0	13	0	150	96
17	52	34	38	0	15	0	14.5	0	15	0	120	120

TABLE 4

Partition of total outflow from each coronary artery

EXPT. NO.	LEFT CIRCUMFLEX ARTERY DRAINING INTO:			ANTERIOR LEFT DESCENDING ARTERY DRAINING INTO:			RIGHT CIRCUMFLEX ARTERY DRAINING INTO:		
	Coronary sinus—%	Right ventricle—%	Left ventricle—%	Coronary sinus—%	Right ventricle—%	Left ventricle—%	Coronary sinus—%	Right ventricle—%	Left ventricle—%
1	70	8	22	65	28	7	7	87	6
2	47	52	1	27	76	2	0	100	0
3	23	43	33	47	37	16	6	82	12
4	40	60	0	41	59	0	0	100	0
5	39	60	1	48	52	0	0	100	0
6	12	77	11	41	57	2	0	89	11
7	15	54	31	47	40	13	0	58	42
8	18	64	18	39	53	8	0	100	0
9	49	29	22	52	38	10	0	100	0
10	43	39	18	25	56	19	0	92	8
11	37	60	3	31	61	8	3	95	2
12	57	35	8	38	51	11	7	91	2
Range...	12–70	8–77	0–33	25–65	28–76	0–19	0–7	58–100	0–42
Av.....	38	48	14	42	51	7	1	92	7

between the right and left hearts in compensating for coronary artery occlusion has been previously discussed by us (6).

Table 4 shows the drainage partition of each of the coronary arteries. Our results are clear in showing the wide variation of drainage in the differ-

ent experiments, and demonstrate beyond doubt the variation in the functional pattern of the coronary system. Our results do not agree with those of Anrep, Blalock and Hammouda (4) as regards the coronary sinus flow. While we obtained values as high as theirs in individual experiments (except in the case of the right circumflex artery), the average percentage in our 12 experiments is about 20 to 30 per cent lower than theirs. This difference, as we have pointed out above, is due to a difference in partition in Morawitz cannula drainage between the beating and fibrillating hearts. Nevertheless, their results erroneously give the idea of a constancy of the coronary flow pattern.

In our experiments, the sinus drains anywhere from 12 to 70 per cent of the flow from the left circumflex artery, the drainage channels of the right heart drain anywhere from 8 to 77 per cent and those of the left anywhere from 0 to 33 per cent. On the average, 38 per cent of the flow of the left circumflex artery drains away via the coronary sinus, 48 per cent via the channels of the right heart and 14 per cent via those of the left heart. In the case of the left anterior descending artery, the coronary sinus drains away 25 to 65 per cent (average 42 per cent), the drainage channels of the right heart, 28 to 76 per cent (average 51 per cent) and those of the left heart, 0 to 19 per cent (average 7 per cent). The left circumflex thus has on the average a greater proportion of its blood emptying into the left heart than the anterior left descending. The drainage of the right circumflex coronary artery is strikingly different from that of the other two branches. The drainage of this branch via the coronary sinus in our 12 animals varied from 0 to 7 per cent (average 1 per cent), and the total absence of sinus drainage was found in 8 out of 12 instances. The difference between our results and those of Anrep et al. (4) who found the sinus drained 33 to 40 per cent of the blood from the right circumflex in 3 beating hearts is due undoubtedly, as already mentioned, to the different redistribution of blood in the beating and fibrillating hearts. In the beating heart without Morawitz cannula the drainage via the coronary sinus would be intermediate between our results and those of Anrep, for the reasons mentioned above. Almost all the blood from the right circumflex in the fibrillating heart goes into the accessory veins and Thebesian channels of the right heart (the proportion varying from 58 to 100 per cent and averaging 92 per cent). While little blood goes into the left ventricle, the average being 7 per cent and in 5 instances 0 per cent, one experiment was found in which 42 per cent of the right circumflex blood drained into the left ventricle; this last is the largest value of drainage into the left heart found for any branch in this series.

Table 4 thus brings out clearly the wide variations in partition of coronary drainage of the three branches and the striking difference in the pattern of drainage from the right as compared with that from the left coro-

nary arteries. The large variability in the part played by the coronary sinus in the drainage of coronary blood is dependent as our results show on *a*, the wide variation in the rôle of the sinus in draining each coronary branch, and *b*, the wide variation in the partition of blood flow between the three coronary branches.

Our results show clearly the absence of any constant pattern of distribution of coronary flow, and brings out some hitherto unsuspected peculiarities. It is obvious that in addition to the variations in the ratio of sinus outflow/total coronary inflow which our previous work (6) has shown occurs under various dynamic circumstances in any beating heart, the present results indicate beyond doubt that the sinus outflow/total coronary inflow ratio varies widely in different individuals of the same species even under like conditions. In the face of such evidence, it is difficult to see how it can be maintained that the coronary sinus flow is a quantitative or even a qualitative measure of the total coronary flow. This being so, it is apparent that coronary sinus flow cannot be used per se to determine the action of drugs, nerves and other factors on the total coronary flow. Results based on coronary sinus flow must await confirmation by more accurate methods. Undoubtedly the inadequacy of the coronary sinus outflow method explains the many contradictions which exist in the literature.

SUMMARY

A method is described for measuring total coronary inflow, drainage from the coronary sinus, drainage from the Thebesian vessels and accessory veins of the right heart, and drainage from the Thebesian vessels and accessory veins of the left heart in an isolated, fibrillating dog's heart with the 3 main coronary branches perfused with defibrinated blood at constant pressure and temperature. The results of 12 experiments are reported.

The proportion of total coronary flow carried by each of the three main coronary arteries (left circumflex, anterior left descending, and right circumflex) was found to be widely variable in different individuals.

Evidence is presented to show the probable existence of functional anastomoses between the three main coronary arteries in some animals.

The proportion of total outflow carried by each of the three drainage channels was found to be widely variable in different individuals. In particular, the proportion drained by the coronary sinus varied from 17 per cent to 44 per cent with an average of 32 per cent. These results are not in accord with the almost constant sinus drainage of 60 per cent reported by Anrep, Blalock and Hammouda.

The differences in coronary sinus drainage between the beating and fibrillating heart are discussed.

The distribution of blood from each of the three main coronary arteries to each of the three drainage channels was found to be widely variable in different individuals.

The invalidity of using coronary sinus outflow as an index of total coronary flow is further substantiated.

We are indebted to the members of the department without whose help these experiments could not have been executed.

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HEART FAILURE ANALYZED IN THE ISOLATED HEART CIRCUIT[1]

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Experimental analysis of the causes and effects of heart failure have given rise to conflicting conclusions. Much of the disparity in experimental results has arisen because of the inadequacies of the various methods used to study heart failure. We have developed a method which eliminates most of the difficulties present in methods previously employed and permits the determination of the work, oxygen consumption, and efficiency of the isolated heart beating under controlled conditions in a single circuit. We have used this preparation to study spontaneous heart failure.

PROCEDURE. First, a classical heart-lung preparation, perfused with defibrinated blood, is established. The general technique used in this laboratory has been previously described (1). Then a cannula connected with the inflow reservoir is tied into the left auricle but not opened, and another cannula leading to an artificial resistance is tied into the central end of the left pulmonary artery. This is also left unopened. As the right pulmonary artery is ligated, the clamps on the left auricular and left pulmonary cannulae are removed. A double circuit is thus established, the right and left heart being fed from the same reservoir and independently pumping blood into the aerating chamber, whence the blood is pumped back into the reservoir. The inflow levels and resistances for each side of the heart are adjusted to the desired levels and each lung is securely tied off at the hilus.

The double circuit is now converted into a single one by connecting the outflow from the aorta beyond the artificial resistance with the cannula in the superior vena cava, which is disconnected from the inflow reservoir. The blood now flows from the reservoir into the left heart where it is pumped through a resistance into the right heart. From here, it is driven by the right ventricle through a resistance into the aerator. A mechanical

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pump forces the blood back into the main inflow reservoir. Inflow and resistances are again adjusted to the desired pressures and outflow. The resistances and mean arterial pressures are read off from mercury manometers and the auricular pressures from water manometers. The outflow from the right heart is measured with a stop watch and graduate by turning a three-way stopcock. The left heart outflow is read in a modified Ludwig stromuhr, previously described (2), which is interposed between an elastic pressure bottle and a peripheral resistance. The diastolic size of the heart is determined indirectly by caliper measurements of cardiac diameters at fixed points or directly by an oncometer. Arterial blood

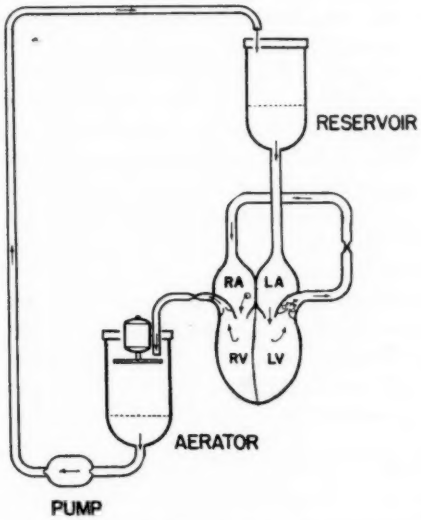

Fig. 1. Diagram of single circuit isolated heart preparation, showing course of blood flow.

samples are obtained from a side-tube in the left heart outflow system, and opened only when taking the sample. Venous blood samples are obtained from a simple glass cannula passed into the coronary sinus via the inferior vena cava. Except when taking samples, the coronary sinus cannula is connected with a cannula in the azygos vein, the blood thus flowing into the superior vena cava and right auricle. This final arrangement of the single circuit isolated heart preparation is shown diagrammatically in figure 1. Detailed diagram and description has been published elsewhere (21).

The readings of the various measurements are made during the course of the experiment at intervals varying from one to twenty minutes, depend-

ing on the rapidity of change, the ease of making the reading, and its importance. Thus, pressures, flows, resistances, and heart rate are read every one to four minutes, diastolic volume and temperature every five to ten minutes. Blood oxygen samples are taken every ten to twenty minutes and blood carbon dioxide, sugar and lactic acid samples only at the beginning and end of the experiment. Blood oxygen content is determined by the method of Van Slyke and Neill (3) in random order. Checks show that the amount of loss of oxygen by diffusion from the blood samples under oil and on ice is less than 0.2 volume per cent. Plasma carbon dioxide analysis is also done by the Van Slyke and Neill (3) technique on blood collected under oil. The blood for sugar and lactic acid is preserved with sodium fluoride. The sugar and lactic acid are determined in duplicate by the Somogyi modification (4) of the Schaffer-Hartmann method and the method of Avery and Hastings (5) respectively.

We are thus able to measure simultaneously (*a*), mean right and left arterial blood pressures; (*b*), right and left arterial resistances; (*c*), mean right and left venous pressures; (*d*), right and left heart outflows; (*e*), heart rate; (*f*), diastolic ventricular volume; (*g*), blood temperature; (*h*), arterio-venous blood oxygen difference of the heart, and (*i*) alteration in the level of blood sugar, lactic acid, and plasma carbon dioxide content during the course of the experiment.

From the above data several other factors can be computed.

1. The total coronary flow (less the insignificant portion draining into the left heart via Thebesian channels (6)) is represented by the difference between aortic and pulmonary flows simultaneously determined.

2. The oxygen consumption of the heart is calculated from the formula

$$\frac{\text{Arterio-venous difference (vol. per cent)} \times \text{coronary flow (cc. per min.)}}{100}$$

3. The work of each ventricle is calculated from the simple formula, $W = QR$, where W is the work in kilograms per hour, Q, the volume flow of blood in liters per hour (equals volume in cubic centimeter per minute $\times$ 0.06) and R, the mean arterial pressure in meters of blood (equals millimeters Hg $\times$ 0.0135). The coronary flow is, of course, added to the aortic flow in determining Q of the left ventricle. The work of the auricles is neglected, the kinetic factor ignored, and no attempt at integration of pressure and volume curves (7) made, since these refinements, at the rates of flows studied and for the purposes in hand, are not necessary. The work of the ventricles determined separately is added together avoiding thereby any assumptions as to the work of the right ventricle, such as have been made in heart-lung preparations and in the intact animal.

4. The efficiency of the heart is calculated as follows: The oxygen consumption of the heart, in liters per hour, is multiplied by 2132.5 (equals

kilograms equivalent of 5 large calories or one liter of oxygen). This is based on the assumption that, at an RQ of 1.00[3], the heart generates 5 large calories for each liter of oxygen consumed, each large calorie being equivalent to 426.5 kilogram-meters of energy. The ratio of the actual mechanical work to the energy equivalent of the oxygen consumption, both expressed in kilogram-meters per hour, represents the mechanical efficiency of the heart.

In these experiments, once adjustments are made to obtain the desired flow and pressure levels, no further readjustments are made except of the aortic and pulmonary resistances, which are varied so as to keep the aortic and pulmonary arterial pressures as constant as possible. In these experiments, any change in left auricular pressure must represent an alteration in the cardiac resistance to inflow since the head of inflow pressure to the left heart is constant. When such resistance in the form of an increase in mean left auricular pressure occurs, there is a decrease in the inflow pressure gradient which will of course be accompanied by a decrease in cardiac inflow and hence in cardiac outflow.

Results. Spontaneous heart failure occurring under these circumstances was completely studied in four preparations. Figure 2 is a detailed graphic protocol of one such experiment. In figure 3, the curves of the pertinent data only, smoothed out by the use of fewer points, are shown for one of the other experiments.

I. The initial state of the preparation. In table 1, the range of variations in the initial values for oxygen consumption, coronary flow, work, and mechanical efficiency are presented. These are of the order, in general, of values reported by other workers (8) demonstrating that the preparation we employed is initially "physiological."

II. Changes in the blood. The carbon dioxide analyses show that the blood was hypocapneic, due to the loss of carbon dioxide to the air. This had no apparent adverse effect on the preparation. The hypocapnea became slightly more marked during the course of the experiment, due in part to a further loss of carbon dioxide to the air and in part to a slight decrease in alkali reserve because of the increase in blood lactic acid. The increase in lactic acid was slight in three experiments, and there was no change in one. There was, however, a moderate decrease in blood sugar in all experiments although not to hypoglycemic levels. The greater change in blood sugar than in lactic acid may have been due in part to the utilization of lactic acid by the heart, as shown by McGinty (9) and Evans et al. (10). Both the blood and the room temperatures were kept constant in these experiments, thus ensuring constancy in the temperature of the

[3] The RQ was not measured directly, although it has been shown that at the blood sugar levels in these experiments, only carbohydrate is utilized (8).

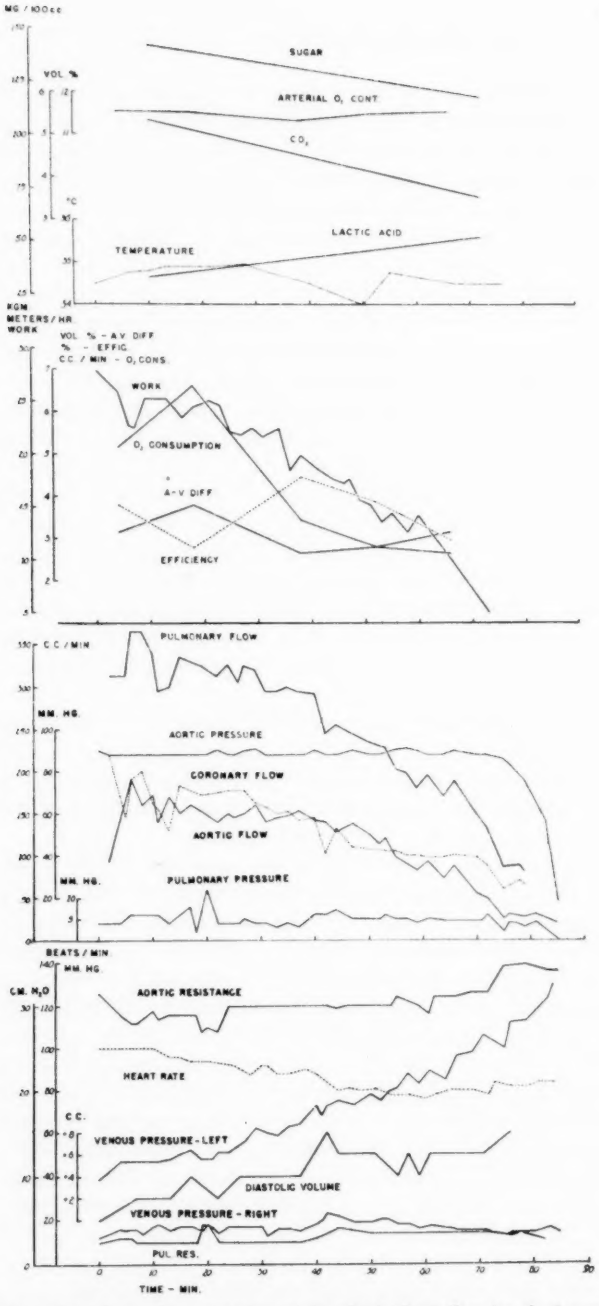

Fig. 2. Complete data of an experiment in which the inflow head of pressure and the aortic and pulmonary arterial pressures are artificially kept constant, showing the changes with progressive left heart failure.

myocardium. The relatively slight changes in oxygen content of the arterial blood indicated that aeration was adequate and hemolysis slight.

III. Changes in circulatory dynamics. The development of cardiac failure was characterized by a series of circulatory changes. Thus, there

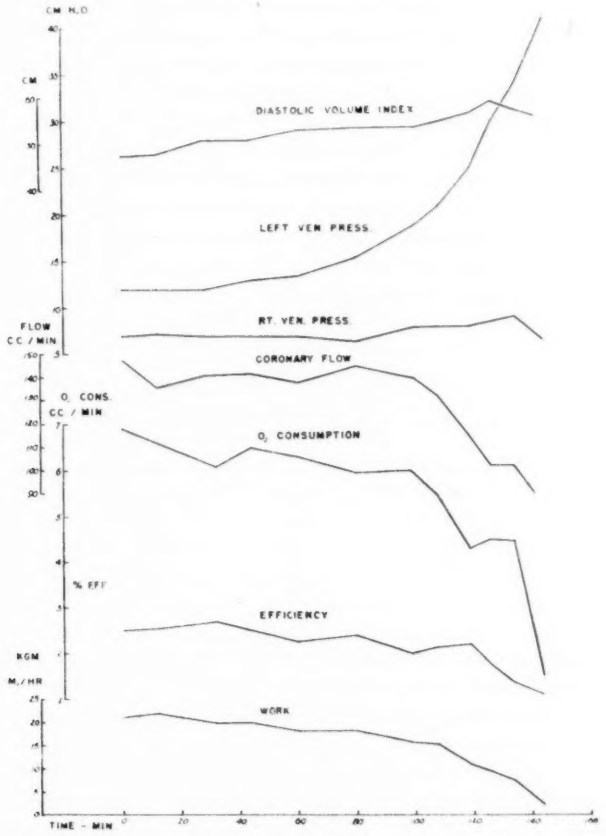

Fig. 3. Pertinent data of an experiment in which the inflow head of pressure and the aortic and pulmonary arterial pressures are artificially kept constant, showing the changes with progressive left heart failure. The curves are smoothed out by the use of fewer points.

was a decrease in pulmonary and aortic flow and a slight decrease in coronary flow even before the mean aortic pressure fell. The mean aortic pressure was usually kept constant by progressively increasing the resistance of the left side. The pulmonary arterial pressure could be kept con-

stant without much change in the resistance of this side. Terminally, it was impossible to maintain aortic pressure by increasing the resistance, and the pressure was therefore permitted to fall progressively. This was accompanied by a further and more definite decrease in coronary flow. There was a progressive and accelerating rise in the left auricular pressure. The right auricular pressure remained relatively unchanged. In one experiment, however, it fell because of the rapid decrease in inflow from the left ventricle. In two experiments, there was a moderate terminal rise in right auricular pressure coincident with the marked decline in coronary flow. The diastolic volume of the entire heart either remained relatively unchanged or became moderately increased.

In some experiments, there were changes in the dynamics before the onset of heart failure, and some of these changes progressed during the course of the experiment. There was usually a slight decrease in heart rate and in one experiment a more marked early decrease, which tapered off during the progress of the experiment. There was always a terminal

TABLE 1

Data at start of experiments

HEART WEIGHT	CORONARY FLOW	OXYGEN CONSUMPTION	WORK	MECHANICAL EFFICIENCY
grams	*cc./min.*	*cc./min.*	*kgm./hr.*	*per cent*
78	80	3.00	17.5	5.3
123	220	5.20	20.5	3.8
148	130	6.85	22.2	2.5
155	96	4.05	19.0	3.7
170	125	6.50	18.4	2.0

more marked decrease in heart rate, followed by such terminal phenomena as extrasystoles and finally ventricular fibrillation. In one experiment, there was a progressive increase in coronary and hence an equivalent decrease in aortic flow before the onset of heart failure, due presumably to a progressive coronary dilatation. As the heart failure progressed in this preparation, however, the coronary flow began to diminish.

IV. Changes in energetics. Failure of the heart was accompanied in all experiments by a progressively accelerated decrease in oxygen consumption. The work of the heart declined pari passu with the oxygen consumption. Since the arterial pressures were kept relatively constant until near the end of the experiment, the important dynamic factor accompanying this decrease was the diminution in cardiac outflow. The mechanical efficiency of the heart, on the other hand, remained relatively unchanged until the end of the experiment when it tended to be decreased.

Discussion. *I. The dynamics of heart failure.* The series of events that takes place as the left ventricle fails in these experiments is as fol-

lows: The left ventricle becomes unable to do the work imposed upon it by the pressure head governing the inflow and by the resistance to its outflow which constitute its load. There is therefore a tendency for the left arterial blood pressure to fall, a tendency which is prevented by artificially increasing the resistance on the left side. This of necessity translates the decrease in work to a decrease in outflow. With this decrease in outflow, the inflow remaining the same, residual blood accumulates in the ventricle, increasing its diastolic size and pressure. This increase in diastolic pressure of the left ventricle constitutes a resistance to inflow from the left auricle, an effect enhanced by such relative insufficiency of the mitral valve as may occur. Blood, therefore, also accumulates in the left auricle, increasing its pressure and volume. In both the ventricle and auricle, the increment of pressure is progressively augmented with each increment of volume, so that the resistance to inflow increases in a geometric ratio. The increase in the mean left auricular pressure by reducing the pressure gradient from the constant pressure head in the reservoir to the auricle reduces the inflow to the left heart. The decrease in inflow produced in this way tends to counterbalance those factors mentioned above which tend to increase the diastolic volume. Since the right heart in our preparation is dependent on the outflow from the left, the decrease in the latter will reduce the size of the right ventricle. The total ventricular volume which is the sum of the volumes of each ventricle will therefore not increase as much as the left ventricular volume. The net effect is therefore no change or occasionally a slight increase in the combined diastolic volume. While the ratio of work to right ventricular diastolic size is probably unchanged, the ratio of work to left ventricular diastolic rise is progressively decreased. Failure of the right ventricle sometimes occurs terminally, due probably to failure of the coronary flow. The same chain of events takes place in this chamber as occurs in left ventricular failure, i.e., an increase in venous pressure, in diastolic volume, and despite this a decrease in outflow, pulmonary arterial pressure and work.

In discussing failure of the heart, it is important to define it in terms of a single chamber rather than the heart as a whole. Failure of a ventricle is characterized by its doing less work at a given diastolic volume, or the same work at a greater diastolic volume. This decrease in the ratio of diastolic volume to work is apparently a mechanical effect of the changes in dynamics caused by the decreased contractile power of the ventricle. Dilatation is therefore an effect of the failure and in its turn a method of compensation which enables the ventricle to continue to perform its work quota. When this compensatory increase in diastolic volume fails to keep pace with the decrease in contractile power of the ventricle, the increase in its load, or both, the ventricle becomes unable to perform its work, and progressive irreversible heart failure ensues.

II. The energetics of heart failure. Since it has been shown that the

heart cannot contract a significant oxygen debt (11), it is justified to assume that oxygen consumption is in direct proportion to the total energy release. It has also been shown that, in the absence of failure, this total energy is a linear function of the work done and hence of the diastolic volume (12). With the development of left ventricular failure in our experiments, despite a relatively unchanged or actually increased diastolic volume, the total energy released diminished progressively. The proportion of this total energy, however, which the heart could convert into mechanical work, remained relatively unchanged, except for a moderate terminal decrease. Thus, heart failure is characterized not only by a decrease in the ratio $\frac{\text{mechanical work done}}{\text{diastolic volume}}$ but also in the ratio $\frac{\text{total energy released}}{\text{diastolic volume}}$. That the latter relationship holds is apparent not only from the foregoing experiments but also in a confirmatory experiment in which the work of the heart was kept constant by increasing the inflow pressure head as the heart failed. The progressive increase in diastolic volume in

TABLE 2

Effect of spontaneous heart failure on energetics of heart when work of heart is kept constant

TIME	DIASTOLIC VOLUME	LEFT VENOUS PRESSURE	RIGHT VENOUS PRESSURE	CORONARY FLOW	O_2 CONSUMPTION	MECHANICAL EFFICIENCY	WORK
	cc.	*cm. H_2O*	*cm. H_2O*	*cc./min.*	*cc./min.*	*per cent*	*kgm/hr*
Onset	C*	9.5	2.5	125	6.5	2.2	18
After 38 minutes	C+12.5	12.2	3.2	148	6.5	2.3	19
After 88 minutes	C+39	25.5	6.8	122	6.5	2.25	18.5

C* = diastolic volume at onset of experiment.

this experiment was accompanied by no significant change in oxygen consumption or mechanical efficiency (see table 2). This relationship between oxygen consumption and diastolic size indicates that the essential change in heart failure is the decline in the ability of the heart to liberate energy at a given diastolic size. This may be due to metabolic changes in each muscle fiber, thus impairing the ability of each fiber to release energy, or it may be caused by the total loss of function of some fibers, thus reducing the number of functioning elements releasing energy.[4] It is possible that both processes are operating. This lessened ability of the heart to release energy is not associated with any loss of its ability to convert the energy released into mechanical work. The efficiency of this conversion is unchanged, and the work remains a constant function of total energy liberated. If the work of the heart is permitted to decline,

[4] This is not due to demonstrable embolism, as cambric cloths of fine mesh (50μ) in the funnel above the reservoir failed to reveal any particles at the end of the experiment, in two preparations similar to those reported here.

the oxygen consumption will decline parallel with it. The work can be kept constant only by virtue of an increase in diastolic volume sufficient to keep the energy liberation of the heart constant. Only terminally may the ability of the heart to convert the total energy liberated into work (mechanical efficiency) be decreased; such a terminal decrease is, of course, accompanied by a marked further diminution in the ability of the heart to release energy.[5]

Our results do not support the contention of Starling and Visscher (12) that the ratio $\frac{\text{oxygen consumption}}{\text{diastolic volume}}$ is the same in heart failure as in the "physiological" heart. Their observations forced these workers to the conclusion that, since the work of the heart decreased with failure, there is a decrease in the efficiency, due both to a progressively smaller numerator and a progressively larger denominator in the equation, efficiency equals $\frac{\text{work}}{\text{oxygen consumption}}$. Peters and Visscher (13), repeating some of the experiments of Starling and Visscher on heart-lung spirometer preparations, kept at constant diastolic volume, found a decrease in oxygen consumption in some experiments. They explained these results by postulating an actual decrease in diastolic size due to myocardial edema. Although edema did occur in some of our experiments, it was frequently slight and could in no wise explain the associated increase in venous pressure observed.

There are several sources of error inherent in the heart-lung spirometer method. Pulmonary edema and vasomotor changes in the pulmonary vessels can alter the dynamics of the right and left heart without their being apparent to the investigator. Variations in the metabolism of the lungs and the development of pulmonary edema can introduce an indeterminable error in the determination of the oxygen consumption of the heart. The pulmonary edema would be especially significant since it occurs during heart failure and thus can mask the true oxygen consumption of the heart at this time. The pulmonary congestion and edema occurring during heart failure also increase the work of the right ventricle, and this will be overlooked unless measured directly. This may be the case in the experiments of Peters and Visscher (13) in which they assume the work of the right ventricle always to be one-sixth of that of the left. These factors, we believe, are responsible for the discrepancy between their results and ours.

There are also sources of error in the isolated heart preparation hitherto employed to study the metabolism of heart failure. The Langendorf preparation is unsuitable for such studies since the left ventricle is not

[5] The terminal decrease in efficiency may be associated here, as in the "physiological" heart (14), with the decrease in work.

pumping blood. Daly (19) used an isolated heart circuit in which each side of the heart received blood separately. This is an artificial separation of the flows of the right and left heart which are physiologically interdependent.[6] This and the marked concentration of the blood in his experiments make his preliminary results of questionable value. Rühl (15) obtained results similar to ours with respect to oxygen consumption and heart failure. In his isolated heart preparation, however, he used histamine to impair the contractile power of the myocardium, a drug which has collateral effects on the coronary arteries and perhaps also on cardiac metabolism. He studied spontaneous heart failure on the heart-lung preparation, in which the use of the Morawitz cannula technique for determining total coronary flow is subject to serious error (cf. 1 and 6).[7] Although our results are in accord with his in respect to the decrease in oxygen consumption in heart failure, we found no increase in coronary flow or in efficiency such as he reported in his heart-lung experiments, in which failure was induced by such drugs as histamine, pernocton, and avertin.

In brief, it is apparent that the loss of mechanical efficiency is not the essence of heart failure, since such a loss does not occur except as a terminal event. Failure of a heart chamber is due either to an increase in load, or a loss in contractile power, or both, to a point where the chamber begins to fail to do the work imposed upon it by the load. The loss of contractile power is manifested by a failure to release energy (as indicated by a decrease in the oxygen consumption) and not by a change in the mechanical efficiency with which this energy is utilized. If, however, dilatation takes place, as it does because of residual blood in the chamber, the energy liberation and hence the work may remain unchanged. As this process progresses, a point is eventually reached when the increase in diastolic size can no longer cope with the load to keep the energy liberation and hence the work constant. The decline in work is now not only due to a sharp decrease in total energy released but also to a terminal decrease in the efficiency of its utilization and leads to a rapid downward progression.

SUMMARY

1. A single isolated heart circuit is described for the study of the dynamics and energetics of spontaneous heart failure.

[6] The same criticism applies to the studies of Mansfeld and Hecht (20) on the Dusser de Barenne preparation.

[7] This criticism applies also to the results of Harrison, et al. (16) who used a Morawitz cannula in the intact anesthetized animal to determine total coronary flow. The use of drugs to produce heart failure in Harrison's experiments raises the question, as in heart-lung preparations (15), (17) and (18), of whether there are side actions of the drug other than those leading to heart failure, which may affect the interpretation of results.

2. It is possible in this preparation to permit failure to occur with little or no change in total diastolic volume.

3. With the development of heart failure, and with a relatively unchanged diastolic volume, there is a progressive decrease in the work and oxygen consumption of the heart and little change in its mechanical efficiency.

4. When the work of the heart is kept constant, no change in oxygen consumption or mechanical efficiency occurs, despite a progressive increase in the diastolic volume and the left auricular pressure.

5. These experiments demonstrate that heart failure must be defined in terms of a single chamber, rather than the heart as a whole.

6. It is concluded that failure of a heart chamber is due to an increase in load, a decrease in contractile power or both of such a degree that the chamber begins to fail to do the work imposed upon it by the load.

7. It is shown that loss of contractile power is manifested by a reduction in total energy release and hence work at a given diastolic volume and (except terminally) not by a decrease with which the liberated energy is utilized for mechanical work.

We are indebted to the members of the department, whose technical assistance was invaluable in performing these experiments.

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